DRAFT RISK ASSESSMENT OF THE POTENTIAL HUMAN HEALTH EFFECTS ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID AND ITS SALTS

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Glossary of Abbreviations

ALT	alanine aminotransferase, serum glutamyl pyruvic transaminase, SGPT
AP	alkaline phosphatase
APFO	ammonium salt of perfluorooctanoic acid
AST	aspartate aminotransferase, serum glutamyl oxaloacetic transaminase, SGOT
AUC	area under the curve
BMI	body mass index
BUN	blood urea nitrogen test
CCG	Clinical Care Groups software
CCK	cholecystokinin-33 (human); cholecystokinin (rat)
CI	confidence interval
Cl	total body clearance
CL_R	renal clearance
ConA	concanavalin A
DEHP	di(2-ethylhexyl)phthalate
DHEAS	dehydroepiandrosterone sulfate
EGF	epidermal growth factor
FMPP	Familial Male-limited Precocious Puberty
FID	flame ionization detector
FSH	follicle stimulating hormone
GD	gestation day
GGT	gamma glutamyl transferase
HDL	high-density lipoprotein
17-HP	17 gamma-hydroxyprogesterone
HPLC/ESMS	high performance liquid chromatography/electrospray tandem mass spectrometry
HRBC	horse red blood cell
HSA	human serum albumin
i.p.	intraperitoneal
i.v.	intravenous
K_d	dissociation constant
LD	lactation day
LDL	low-density lipoprotein
LH	luteinizing hormone
LLOQ	lower limit of quantitation
LOAEL	lowest-observed-adverse-effect level
LD50	lethal dose 50%
LPS	lipopolysaccharide

M570	N-methyl perfluorooctanesulfonamidoacetate
MOA	mode of action
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
OAT	organic anion transporter
OPPT	Office of Pollution Prevention and Toxics
PACT	pancreatic acinar cell tumors
PFHS	perfluorohexanesulfonate
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFC	plaque forming cell immune function test
PPARα	peroxisome proliferator-activated receptor α
ppb	parts per billion
ppm	parts per million
RREpC	relative risk ratio for each episode of care
RSA	rodent serum albumin
SEM	standard error of the mean
SGOT	serum glutamyl oxaloacetic transaminase, AST
SGPT	serum glutamyl pyruvic transaminase, ALT
SHBG	sex hormone-binding globulin
SIR	standardized incidence ratio
SMR	standardized mortality ratio
$t_{1/2}$ or $T_{1/2}$	half life
TSH	thyroid-stimulating hormone
Vd	volume of distribution

Executive Summary

As part of the effort by the Office of Pollution Prevention and Toxics (OPPT) to understand health and environmental issues presented by fluorochemicals in the wake of unexpected toxicological and bioaccumulation discoveries with respect to perfluorooctane sulfonates (PFOS), OPPT has been investigating perfluorooctanoic acid (PFOA) and its salts. PFOA and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses. PFOA and its salts are persistent in the environment. Most of the toxicology studies have been conducted with the ammonium salt of perfluorooctanoic acid, which is referred to as APFO in this report.

Human Health Effects

Epidemiological studies on the effects of PFOA in humans have been conducted on workers. Most of the studies were cross-sectional and focused primarily on males. Developmental outcomes have not been studied. A retrospective cohort mortality study demonstrated a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures PFOA. However, in an update to this study in which more specific exposure measures were used, a significant association for prostate cancer was not observed. Other mortality studies lacked adequate exposure data which could be linked to health outcomes. A study which examined hormone levels in workers reported an increase in estradiol levels in workers with the highest PFOA serum levels; however, these results may have been confounded by body mass index. Cholesterol and triglyceride levels in workers were positively associated with PFOA exposures, which is inconsistent with the hypolipidemic effects observed in rat studies. A statistically significant positive association was reported for PFOA and T3 levels in workers but not for any other thyroid hormones.

Little information is available concerning the pharmacokinetics of PFOA and its salts in humans. An ongoing 5-year, half-life study in 7 male and 2 female retired workers has suggested a mean serum PFOA half-life of 4.37 years (range, 1.50 – 13.49 years). Gender differences in elimination of PFOA have not been observed in humans based on the limited data available in the half-life study in retired workers. Metabolism and pharmacokinetic studies in non-human primates are limited to a study of 3 male and 3 female cynomolgus monkeys administered a single i.v. dose of 10 mg/kg potassium PFOA. In male monkeys, the average serum half-life was 20.9 days. In female monkeys, the average serum half-life was 32.6 days.

Studies in adult rats have shown that the ammonium salt of PFOA (APFO) is absorbed following oral, inhalation and dermal exposure. Serum pharmacokinetic parameters and the distribution of PFOA has been examined in the tissues of adult rats following administration by gavage and by i.v. and i.p. injection. PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats.

There are gender differences in the elimination of PFOA in adult rats following administration by gavage and by i.v. and i.p.injection. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8 to 16 hours,

while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138 to 202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

Several recent studies have been conducted to examine the kinetics of PFOA in the developing Sprague-Dawley rat. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. During lactation and for the first several weeks after weaning, the elimination of PFOA is similar in males and females pups. Between 4-5 weeks of age, the elimination in male rats assumes the adult pattern and the gender difference becomes readily apparent. Distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

In acute toxicity studies in animals, the oral LD50 values for CD rats were >500 mg/kg for males and 250-500 mg/kg for females, and <1000 mg/kg for male and female Wistar rats. There was no mortality following inhalation exposure of 18.6 mg/L for one hour in rats. The dermal LD50 in rabbits was determined to be greater than 2000 mg/kg. APFO is a primary ocular irritant in rabbits, while the data regarding potential skin irritancy are conflicting.

APFO is not mutagenic. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation. APFO did not induce gene mutation when tested with or without metabolic activation in the K-1 line of Chinese hamster ovary (CHO) cells in culture. APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in C_3H $10T_{½}$ mouse embryo fibroblasts and in the mouse micronucleus assay.

Repeat-dose studies have been conducted in non-human primates. In a 13-week study with Rhesus monkeys, exposure to doses of 30 mg/kg-day or higher resulted in death. Clinical signs of toxicity were noted at doses as low as 3 mg/kg-day. Unlike rodent studies, analyses of the serum and liver levels did not reveal a gender difference in monkeys, but the sample size was very small. In a 6-month study of male cynomolgus monkeys, there was a steep dose response curve for mortality. Increases in liver weight were noted at doses as low as 3 mg/kg-day, but there was no evidence of peroxisome proliferator-activated receptor alpha activity (PPARα).

Repeat-dose studies in rats and mice demonstrated that the liver is the primary target organ. Due to gender differences in elimination, adult male rats exhibit effects at lower administered doses than adult female rats. Dietary exposure to APFO for 90 days resulted in significant increases in liver weight and hepatocellular hypertrophy in female rats at 1000 ppm (76.5 mg/kg-day) and in male rats at doses as low as 100 ppm (5 mg/kg-day). Chronic dietary exposure of rats to 300 ppm(males, 14.2 mg/kg-day; females, 16.1 mg/kg-day) APFO for 2 years resulted in increased liver weight, hepatocellular hypertrophy, hematological effects, and testicular masses in males;

and reductions in body weight and hematological effects in females.

The carcinogenic potential of PFOA has been investigated in two dietary carcinogenicity studies in rats. Under the conditions of these studies, there is some evidence that PFOA is carcinogenic, inducing liver tumors, Leydig cell tumors (LCT), and pancreatic acinar cell tumors (PACT) in male Sprague-Dawley rats. The evidence for mammary fibroadenomas in the female rats is equivocal since the incidences were comparable to some historical background incidences. There is sufficient evidence to indicate that PFOA is a PPARα-agonist and that the liver carcinogenicity (and toxicity) of PFOA is mediated by binding to the PPARα in the liver. A mode of action analysis has demonstrated that the hepatic effects are due to PPARα-agonism, and that this mode of action is unlikely to occur in humans. The mode(s) of action for the Leydig cell and pancreatic acinar cell tumors have been investigated, but there is insufficient evidence to link these modes of action to PPARα. The LCT and PACT induced in the rat by PFOA probably do not represent a significant cancer hazard for humans because of quantitative differences in the expressions of LH and CCK_A receptors and of other toxicodynamic differences between the rat and the human. Based on no adequate human studies and uncertain relevance of the tumors from the rat studies, PFOA may be best described as "suggestive evidence of carcinogenicty, but not sufficient to assess human carcinogenic potential" under the draft 1999 EPA Guidelines for Carcinogen Risk Assessment.

PFOA appears to be immunotoxic in mice. Feeding C57Bl/6 mice a diet containing 0.02% PFOA resulted in adverse effects to both the thymus and spleen. In addition, this feeding regimen resulted in suppression of the specific humoral immune response to horse red blood cells, and suppression of splenic lymphocyte proliferation. The suppressed mice recovered their ability to generate a humoral immune response when they were fed a diet devoid of PFOA. Studies using transgenic mice showed that the PPARα was involved in causing the adverse effects to the immune system.

There was no evidence of prenatal developmental toxicity in rats after oral exposure to doses as high as 150 mg/kg-day. Maternal toxicity was seen at 100 mg/kg-day. In a rabbit oral prenatal developmental toxicity study there was a significant increase in skeletal variations after exposure to 50 mg/kg-day APFO. There was no evidence of maternal toxicity at 50 mg/kg-day, the highest dose tested.

A variety of endpoints were evaluated throughout different lifestages in a two-generation reproductive toxicity study in rats exposed to 0, 1, 3, 10, and 30 mg/kg/day APFO. In that study, a reduction in F1 pup mean body weight on a litter basis was observed during lactation (sexes combined) in the 30 mg/kg-day group. F1 male pups in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. F1 female pups in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. Reproductive indices were not affected in the F1 animals. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 male and female pups in the 30 mg/kg-day group. No effects were observed in the F2 pups. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation. Adult systemic toxicity consisted of reductions in body weight in both the F0 and F1 animals.

Human Biomonitoring

While the environmental concentrations and pathways of human exposure to PFOA and its salts are unknown, there are data on PFOA serum levels in workers and the general population. PFOA has been measured in the serum of workers occupationally exposed to perfluorinated chemicals for many years. PFOA has also been detected recently in the serum of the general U.S. population, but at much lower levels than those reported in occupational biomonitoring studies. Individual blood serum samples from 3 separate non-occupational cohorts have been analyzed for PFOA. Cohorts of adults (n= 645) and children (n=598) from various geographic areas of the U.S. and an elderly cohort from Seattle (n = 238) have indicated consistent mean serum levels of PFOA (approximately 5 ng/ml or 5 ppb). The reports indicate that serum levels for most of the individuals in these samples are below 10 ng/ml; however, some of the levels are as high as 56 ng/ml, indicating that a small number of individuals are being exposed at higher concentrations than the rest of the general population.

Risk Assessment and Uncertainties

A margin of exposure (MOE) approach can be used to describe the potential for human health effects associated with exposure to a chemical. The MOE is calculated as the ratio of the NOAEL or LOAEL for a specific endpoint to the estimated human exposure level. The specific endpoint may be from an epidemiology study or an animal toxicology study. The MOE does not provide an estimate of population risk, but simply describes the relative "distance" between the exposure level and the NOAEL or LOAEL. In this risk assessment there is no information on the sources or pathways of human exposure. However, serum levels of PFOA, which are a measure of cumulative exposure, were available from human biomonitoring studies. In addition, serum levels of PFOA were available for many of the animal toxicology studies or there was sufficient pharmacokinetic information to estimate serum levels. Thus, in this assessment internal doses from animal and human studies were compared; this is somewhat analogous to a MOE approach which uses external exposure estimates.

The results of existing epidemiology studies are not adequate for use in quantitative risk assessment, and therefore the analysis was restricted to endpoints in the animal toxicology studies. MOEs were calculated for the general U.S. population. Although some serum level data were available for workers, the data were not adequate to calculate MOEs for occupational exposures. In general, the mean serum levels following occupational exposures appear to be orders of magnitude higher than observed in the general population. Thus, MOEs for workers would be expected to be much less than for the general population.

A variety of endpoints from the animal toxicology studies were used to calculate MOEs for this draft risk assessment. The endpoints encompassed different species, gender and life stages. For this draft assessment, specific recommendations on the most appropriate endpoint/lifestage/species/gender have not been made; rather, all have been presented to provide transparency.

For adults, two sets of MOEs were calculated based on the toxicology studies in non-human primates and rats. First, MOEs were calculated from the cynomolgus monkey study and are based on increased liver weight and possible mortality. The MOE using the geometric mean for the human serum level is 16,739 (8,191 for the 90th percentile). Second, MOEs were calculated from the adult rat studies and are based on reductions in body weight. MOEs were calculated separately for the female and male rat due to the gender differences in pharmacokinetics in this

species. MOEs were calculated by dividing the AUC in the adult female rat by the AUC for the adult humans which is 398 (195 for the 90th percentile) and by dividing the AUC for the adult male rat by the AUC for the adult humans which is 9158 (4481 for the 90th percentile).

MOEs were calculated for the developmental effects in the two-generation reproductive toxicity study in rats. These effects were observed at various times during the maturation of the F1 pups. For both F1 males and females there were reductions in body weight during lactation; significant increases in mortality during the first few days after weaning; and significant delays in the timing of sexual maturation. Mean body weights were also significantly reduced in the time period prior to sexual maturation in both the F1 males and females. The critical period of exposure for each of the effects is not known. For example, it is not known whether prenatal and/or lactational exposure is important for the reduced body weight that was observed during lactation. Similarly, it is not known whether the reduced body weight, mortality, or delayed sexual maturation that occurred during the postweaning period are due to prenatal, lactational, and/or postweaning exposures. Ideally, MOEs would be calculated for each of these exposure periods; however MOEs were not calculated for the lactation period due to uncertainties in pharmacokinetics.

For the prenatal period, MOEs were calculated for the pregnant human female. MOEs were not calculated for the fetus since there is no information on human serum levels in fetuses. MOEs were calculated using both C_{max} and AUC; the MOE based on C_{max} is 3,095 (1548 for the 90th percentile) and the MOE based on the AUC is 823 (412 for the 90th percentile).

For the postweaning period, MOEs were calculated for several endpoints including reductions in body weight, mortality and delayed sexual maturation. These MOEs were based on the geometric mean for children and range from 10,484 - 78,546 (the range using the 90th is 6,044 - 45,279).

This assessment has provided a range of MOEs for several life stages. Several uncertainties have been discussed in a qualitative fashion in the assessment, which highlight the need to interpret the MOEs with caution. For example, MOEs were not calculated for the lactation period due to insufficient data, although this may represent an important exposure period. Similarly, the biomonitoring data for the children are from samples collected in 1994 and may not be representative of current children's serum levels. Finally, there is some uncertainty associated with the determination of the adequacy of a specific MOE in protecting human health in the present context. Traditionally, MOEs are calculated from administered dose levels and estimates of human exposure. In this assessment, the MOEs were calculated from internal dose metrics in animals and humans. While use of internal dose metrics reduces many uncertainties pertaining to exposure, there is little experience or guidance on the factors that should be considered in making judgements about the level of concern associated with a given MOE. Approaches that are used for conventional MOEs, if applied unchanged, indicate that among the populations of interest some individuals are highly exposed, for reasons not understood at this time. However, if conventional approaches for determining levels of concern are not appropriate for MOEs based on internal dose metrics, then this conclusion would have to be re-evaluated as the understanding of this question evolves.

1.0 Scope of the Assessment

As part of the effort by the Office of Pollution Prevention and Toxics (OPPT) to understand health and environmental issues presented by fluorochemicals in the wake of unexpected toxicological and bioaccumulation discoveries with respect to perfluorooctane sulfonates (PFOS), OPPT has been investigating perfluorooctanoic acid and its salts (PFOA). PFOA and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses.

OPPT released a preliminary *Draft Hazard Assessment of Perfluorooctanoic Acid and Its Salts*, dated February 20, 2002, on March 28, 2002, and issued a minor correction to that document on April 15, 2002. That draft assessment indicated that PFOA and its salts are persistent in the environment and in humans with a half life of years. The assessment noted the potential systemic toxicity and carcinogenicity associated with the ammonium salt of PFOA (APFO), which has been the focus of the animal toxicology studies, and observed that blood monitoring data suggested widespread exposure to the general population, albeit at low levels. The Agency has since received considerable additional animal toxicology data on APFO that suggest a potential for developmental/reproductive toxicity and immunotoxicity, and additional human biomonitoring data that indicate low level exposures to the general population that cannot be explained at this time.

On September 27, 2002, the Director of OPPT issued a memorandum announcing that OPPT would initiate a priority review to determine whether PFOA and its salts meets the criteria for action under section 4(f) of the Toxic Substances Control Act. As part of the priority review, the hazard assessment was revised and released on September 30, 2002. Another revision was then released November 4, 2002. In addition, OPPT released a *Preliminary Risk Assessmment of the Developmental Toxicity of PFOA and Its Salts*, dated March 4, 2003. Although there is a wide range of toxicological endpoints associated with exposure to APFO, the initial scope of the preliminary risk assessment focused only on the endpoints that are included in section 4(f); these include cancer, mutations, and birth defects. OPPT did not include gene mutations in the preliminary risk assessment since APFO is not known to be mutagenic. In addition, APFO is a peroxisome proliferator-activated receptor α (PPARα) agonist and through this mode of action could lead to the formation of liver tumors in rodents. The relevance of this mode of action for humans is currently under scientific debate, and the Agency was engaged in activities to resolve this issue. Therefore, the preliminary risk assessment was narrowly restricted to examine only the potential risks of developmental toxicity.

Since the release of the preliminary risk assessment, several pieces of information have become available. Several uncertainties regarding the pharmacokinetics of PFOA during development were discussed in the preliminary risk assessment, and studies have now been conducted to address these uncertainties. In addition, activities aimed at addressing the uncertainties associated with the PPARα-agonist mode of action have made significant progress, and a draft Proposed OPPTS Science Policy paper was presented to the FIFRA Science Advisory Panel on December 9, 2003. This new information has enabled OPPT to extend the assessment activities such that this *draft Risk Assessment of the Potential Human Health Effects Associated With Exposure to PFOA and Its Salts* considers all toxicological endpoints to the extent possible.

The relevant information pertaining to chemical properties, epidemiology, pharmacokinetics and

metabolism, animal toxicology studies, and human exposure have been included in this draft risk assessment. Other information pertaining to ecotoxicity, production and uses, fate and transport, and environmental monitoring are avilable in the *Draft Hazard Assessment of Perfluorooctanoic Acid and Its Salts*, dated November 4, 2002.

2.0 Chemical Identity

Chemical Name: Perfluorooctanoic Acid
Molecular formula: C8 H F15 O2

Structural formula: F-CF2-CF2-CF2-CF2-CF2-CF2-CF2-C(=O)-X,

The free acid and some common derivatives have the following CAS numbers:
The perfluorooctanoate anion does not have a specific CAS number.

Free Acid	(X = OM+; M = H)	[335-67-1]
Ammonium Salt	(X = OM+; M = NH4)	[3825-26-1]
Sodium Salt	(X = OM+; M = Na)	[335-95-5]
Potassium Salt	(X = OM+; M = K)	[2395-00-8]
Silver Salt	(X = OM+; M = Ag)	[335-93-3]
Acid Fluoride	(X = F)	[335-66-0]
Methyl Ester	(X = CH3)	[376-27-2]
Ethyl Ester	(X = CH2-CH3)	[3108-24-5]

Synonyms: 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-PFOA

2.1 Physicochemical Properties

PFOA is a completely fluorinated organic acid. The typical structure has a linear chain of eight carbon atoms. The physical chemical properties noted below are for the free acid, unless otherwise stated. The data for the free acid, pentadecafluorooctanoic acid [335-67-1], is the most complete. The reported vapor pressure of 10 mm Hg appears high for a low melting solid when compared to other low melting solids (chloroacetic acid: solid; MP = 61 to 63 °C; BP = 189 °C; VP = 0.1 kPa [0.75 mm Hg] @ 20 °C; NIOSH, 1994), but is consistent with other perfluorinated compounds with similar boiling points (perfluorobutanoic acid BP = 120 °C, VP 10 mm Hg @ 20 °C; Boit, 1975). Another explanation may be that the 10 mm vapor pressure was measured at an elevated temperature (but the temperature inadvertently omitted), as perfluorooctanoic acid is typically handled as a liquid at 65 °C (3M, 2001). The free acid is expected to completely dissociate in water, leaving the anionic carboxylate in the water and the perfluoroalkyl chain on the surface. In aqueous solutions, individual molecules of PFOA anion loosely associate on the water surface and partition between the air / water interface. Several reports note that PFOA salts self-associate at the surface, but with agitation they disperse and micelles form at higher concentrations (Simister et al., 1992; Calfours and Stilbs, 1985; Edwards et al., 1997). Water solubility has been reported for PFOA, but it is unclear whether these values are for a microdispersion of micelles, rather than true solubility. Due to these same surface-active properties of PFOA, and the test protocol for the OECD shake flask method, PFOA is anticipated to form multiple layers in octanol/water, much like those observed for PFOS. Therefore, an n-octanol/water partition coefficient cannot be determined.

The available physicochemical properties for the PFOA free acid are:

Molecular weight: 414 (Boit, 1975)
Melting point: 45 – 50 °C (Boit, 1975)
Boiling point: 189 – 192 °C / 736 mm Hg (Boit, 1975)
Vapor pressure: 10 mm Hg @ 25 °C (approx.) (Exfluor, 1998)
Water solubility: 3.4 g/L (telomeric [MP = 34 °C ref. 0.01 - 0.02 mol/L ~4 - 8 g/L) (MSDS from Merck, and Fischer, 2003)
pKa: 2.5 (Gilliland, 1992)
pH (1g/L): 2.6 (MSDS Merck)

The PFOA derivative of greatest concern and most wide spread use is the ammonium salt (APFO; CAS No. 3825-26-1). The water solubility of APFO has been inconsistently reported. One 3M study reported the water solubility of APFO to be > 10%. It was noted in an earlier study that at concentrations of 20 g/L, the solution "gelled" (Welter, 1979). These numbers seem surprisingly low for a salt in light of Apollo Scientific selling a 31% aqueous solution of APFO. Reported values for estimated partition coefficient (log Pow) of APFO do not agree. The anticipated formation of an emulsified layer between the octanol and water surface interface would make determination of log Kow impossible.

Determination of the vapor pressure of APFO is complicated. A vapor pressure of 7×10^{-5} mm Hg at 20 °C has been reported for APFO; however, this appears to be too low for a material that sublimates as the ammonium salt (Wolter, 1993). The ammonium salt begins to sublimate at 130 °C. As the temperature increases, 20% of the sample weight of APFO is lost by 169 °C. Other salts (Cs, K, Ag, Pb, Li) do not demonstrate similar weight loss until 237 °C or higher (Lines and Sutcliffe, 1984). Decomposition of different salts produces perfluoroheptene (loss of metal fluoride and carbon dioxide). This occurs at 320 °C for the sodium salt and at 250-290 °C for the silver salt (Boit, 1975).

3.0 Hazard Characterization

3.1 Epidemiology Studies

3M and Dupont have conducted several epidemiology and medical surveillance studies of the workers at their plants in various cities of the U.S. No remarkable health effects that can be directly attributed to PFOA exposure have been reported in fluorochemical production workers described in the studies below. (Serum PFOA concentrations in workers volunteering in these biomonitoring programs are presented in Table 24, Section 4.1.1 and Table 25, Section 4.1.2).

3.1.1 Mortality and Cancer Incidence Studies in Workers

A retrospective cohort mortality study was performed on employees at the 3M Cottage Grove, MN plant which produces APFO (Gilliland and Mandel, 1993). At this plant, APFO production was limited to the Chemical Division. The cohort consisted of workers who had been employed at the plant for at least 6 months between January 1947 and December 1983. Death certificates of all of the workers were obtained to determine cause of death. There was almost complete follow-up (99.5%) of all of the study participants. The exposure status of the workers was categorized based on their job histories. If they had been employed for at least 1 month in the Chemical Division, they were considered exposed. All others were considered to be not exposed to PFOA. The number of months employed in the Chemical Division provided the cumulative exposure measurements. Of the 3537 (2788 men and 749 women) employees who participated in this study, 398 (348 men and 50 women) were deceased. Eleven of the 50 women and 148 of

the 348 men worked in the Chemical Division, and therefore, were considered exposed to PFOA.

Standardized Mortality Ratios (SMRs), adjusted for age, sex, and race were calculated and compared to U.S. and Minnesota white death rates for men. For women, only state rates were available. The SMRs for males were stratified for 3 latency periods (10, 15, and 20 years) and 3 periods of duration of employment (5, 10, and 20 years).

For all female employees, the SMRs for all causes and for all cancers were less than 1. The only elevated (although not significant) SMR was for lymphopoietic cancer, and was based on only 3 deaths. When exposure status was considered, SMRs for all causes of death and for all cancers were significantly lower than expected, based on the U.S. rates, for both the Chemical Division workers and the other employees of the plant.

In all male workers at the plant, the SMRs were close to 1 for most of the causes of death when compared to both the U.S. and the Minnesota death rates. When latency and duration of employment were considered, there were no elevated SMRs. When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI .55 - 4.59). This was based on 4 deaths (1.97 expected). There was also a statistically significant ($p = 0.03$) association with length of employment in the Chemical Division and prostate cancer mortality. Based on the results of proportional hazard models, the relative risk for a 1-year increase in employment in the Chemical Division was 1.13 (95% CI 1.01 to 1.27). It rose to 3.3 (95% CI 1.02 -10.6) for workers employed in the Chemical Division for 10 years when compared to the other employees in the plant. The SMR for workers not employed in the Chemical Division was less than expected for prostate cancer (.58).

An update of this study was conducted to include the death experience of employees through 1997 (Alexander, 2001a). The cohort consisted of 3992 workers. The eligibility requirement was increased to 1 year of employment at the Cottage Grove plant, and the exposure categories were changed to be more specific. Workers were placed into 3 exposure groups based on job history information: definite PFOA exposure (n = 492, jobs where cell generation, drying, shipping and packaging of PFOA occurred throughout the history of the plant); probable PFOA exposure (n = 1685, other chemical division jobs where exposure to PFOA was possible but with lower or transient exposures); and not exposed to fluorochemicals (n = 1815, primarily non-chemical division jobs).

In this new cohort, 607 deaths were identified: 46 of these deaths were in the PFOA exposure group, 267 in the probable exposure group, and 294 in the non-exposed group. When all employees were compared to the state mortality rates, SMRs were less than 1 or only slightly higher for all of the causes of death analyzed. None of the SMRs were statistically significant at $p = .05$. The highest SMR reported was for bladder cancer (SMR = 1.31, 95% CI = 0.42 – 3.05). Five deaths were observed (3.83 expected).

A few SMRs were elevated for employees in the definite PFOA exposure group: 2 deaths from cancer of the large intestine (SMR = 1.67, 95% CI = 0.02 – 6.02), 1 from pancreatic cancer (SMR = 1.34, 95% CI = 0.03 – 7.42), and 1 from prostate cancer (SMR = 1.30, 95% CI = 0.03 – 7.20). In addition, employees in the definite PFOA exposure group were 2.5 times more likely to experience cerebrovascular disease mortality (5 deaths observed, 1.94 expected; 95% CI = 0.84 – 6.03).

In the probable exposure group, 3 SMRs were elevated: cancer of the testis and other male genital organs (SMR = 2.75, 95% CI = 0.07 – 15.3); pancreatic cancer (SMR = 1.24, 95% CI = 0.45 – 2.70); and malignant melanoma of the skin (SMR = 1.42, 95% CI = 0.17 – 5.11). Only 1, 6, and 2 cases were observed, respectively. The SMR for prostate cancer in this group was 0.86 (95% CI = 0.28 – 2.02) (n = 5).

There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs. Four cases were observed and only 1.89 were expected (95% CI = 0.58 – 5.40).

It is difficult to interpret the results of the prostate cancer deaths between the first study and the update because the exposure categories were modified in the update. Only 1 death was reported in the definite exposure group and 5 were observed in the probable exposure group. All of these deaths would have been placed in the chemical plant employees exposure group in the first study. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported. This is important because even 1 prostate cancer death in the definite PFOA exposure group resulted in an elevated SMR for the group. Therefore, if any of the employees' exposures were misclassified, the results of the analysis could be altered significantly. This issue has become more apparent, given the results of a biomonitoring study that took place at the Cottage Grove plant in 2000 in which PFOA concentrations were not correlated with years worked in the Chemical Division but instead were associated with the specific area of the plant where APFO was produced (Olsen, et al., 2003f).

The excess mortality in cerebrovascular disease noted in employees in the definite exposure group was further analyzed based on number of years of employment at the plant. Three of the 5 deaths occurred in workers who were employed in jobs with definite PFOA exposure for more than 5 years but less than 10 years (SMR = 15.03, 95% CI = 3.02 – 43.91). The other 2 occurred in employees with less than 1 year of definite exposure. The SMR was 6.9 (95% CI = 1.39 – 20.24) for employees with greater than 5 years of definite PFOA exposure. In order to confirm that the results regarding cerebrovascular disease were not an artifact of death certificate coding, regional mortality rates were used for the reference population. The results did not change. When these deaths were further analyzed by cumulative exposure (time-weighted according to exposure category), workers with 27 years of exposure in probable PFOA exposed jobs or those with 9 years of definite PFOA exposure were 3.3 times more likely to die of cerebrovascular disease than the general population. A dose-response relationship was not observed with years of exposure.

It is difficult to compare the results of the first and second mortality studies at the Cottage Grove plant since the exposure categories were modified. Although the potential for exposure misclassification was certainly more likely in the first study, it may still have occurred in the update as well. It is difficult to judge the reliability of the exposure categories that were defined without measured exposures. Although serum PFOA measurements were considered in the exposure matrix developed for the update, they were not directly used. In the second study, the chemical plant employees were sub-divided into PFOA-exposed groups, and the film plant employees essentially remained in the "non-exposed" group. This was an effort to more accurately classify exposures; however, these new categories do not take into account duration of exposure or length of employment. Another limitation to this study is that 17 death certificates were not located for deceased employees and therefore were not included in the study. The inclusion or exclusion of these deaths could change the analyses for the causes of death that had a small number of cases. Follow-up of worker mortality at Cottage Grove (and

Decatur) needs to continue. Although there were more than 200 additional deaths included in this analysis, it is a small number and the cohort is still relatively young. Given the results of studies on fluorochemicals in both animals and humans, further analysis is warranted.

Limited data are available on mortality and cancer incidence at Dupont's Washington Works Plant in Parkersburg, WV. These studies were periodically undertaken as part of a medical surveillance program at the plant. The most recent report is summarized here. Cancer incidence for active employees was reported for the 1959-2001 time period and mortality data were reported for active and retired employees for 1957 through 2000 (Dupont, 2003). No other data, such as employee exposure information, lifestyle factors, employee demographics, or other chemicals used at the plant, are available in this report.

In the cancer incidence report, cancer cases were identified through a combination of company health and life insurance claims and company cancer and mortality registries. Standardized incidence ratios (SIRs) were only calculated for those cancers for which 5 or more cases were observed, which included 14 types of cancer. Two of those cancer types were elevated and statistically significant ($p = 0.05$): bladder [SIR = 1.9; 95% CI (1.15-3.07)] and kidney and urinary organs [SIR = 2.3 (95% CI = 1.36-3.65)]. All of the reported cases were male. Some other cancer types with elevated SIRs but which were not statistically significant at $p = 0.05$ included myeloid leukemia (2.02), cancer of the larynx (1.77), multiple myeloma and immunoproliferative (1.72), malignant melanoma of skin (1.3), testicular cancer (1.46), and brain cancer (1.2).

In the mortality report, when all causes of death were reported, SMRs, adjusted for age and gender, were statistically significant ($p = 0.05$) for rheumatic heart disease (SMR = 3.55; 95% CI, 1.14-8.30) and atherosclerosis and aneurysm (SMR = 1.98; 95% CI, 1.17-3.14).

Two separate analyses of leukemia incidence were conducted prior to the above studies at Dupont's Washington Works plant (Walrath and Burke, 1989; Karns and Fayerweather, 1991). The initial study reported a statistically significant ($p = 0.10$) elevated odds ratio (OR) of 2.1 for leukemia incidence for male employees working at the plant from 1956-1989. Eight cases (all male) of different types of leukemia were identified. The OR remained elevated when the workers were divided into wage and salaried employees (2.2 and 2.0, respectively). In the follow-up case-control study, four controls were selected from the plant for each case, matched on gender, age and payroll status. Matched odds ratios were significantly elevated ($p = 0.10$) for employees who had previously worked as custodians and engineers, 8.0 (90% CI, 1.1-60.0) and 7.9 (90% CI, 1.0 - 76.0), respectively and remained elevated (although not statistically significant) for these same job categories within the plant (OR= 4.0 and 5.1, respectively). Matched OR were also reported based on the area of the plant where the cases worked; however, no statistically significant ($p = 0.10$) OR were reported.

The mortality data reported above do not show any statistically significant ($p = 0.10$) elevations in leukemia deaths (all of the cases in the case-control study were dead at the time of the mortality report), possibly because the number of cases was very small and divided among different types of leukemias. The Washington Works data provide some insight as to where more medical surveillance should be concentrated at this plant but provide little information about the relationship of PFOA to mortality or cancer incidence since no exposure information, use of other chemicals, or lifestyle information was collected on these employees.

3.1.2 Hormone Study in Male Workers

Endocrine effects have been associated with PFOA exposure in animals; therefore, medical surveillance data, including hormone testing, from male employees only of the Cottage Grove, Minnesota plant were analyzed (Olsen et al., 1998a). PFOA serum levels were obtained for volunteer workers in 1993 (n = 111) and 1995 (n = 80). Sixty-eight employees were common to both sampling periods. In 1993, the range of PFOA was 0-80 ppm and 0-115 ppm in 1995 using thermospray mass spectrophotometry assay. Eleven hormones were assayed from the serum samples. They were: cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, follicle stimulating hormone (FSH), 17 gamma-hydroxyprogesterone (17-HP), free testosterone, total testosterone, luteinizing hormone (LH), prolactin, thyroid-stimulating hormone (TSH) and sex hormone-binding globulin (SHBG). Employees were placed into 4 exposure categories based on their serum PFOA levels: 0-1 ppm, 1- < 10 ppm, 10- < 30 ppm, and >30 ppm. Statistical methods used to compare PFOA levels and hormone values included: multivariable regression analysis, ANOVA, and Pearson correlation coefficients.

PFOA was not highly correlated with any of the hormones or with the following covariates: age, alcohol consumption, body mass index (BMI), or cigarettes. Most of the employees had PFOA serum levels less than 10 ppm. In 1993, only 12 employees had serum levels > 10 ppm, and 15 in 1995. However, these levels ranged from approximately 10 ppm to over 114 ppm. There were only 4 employees in the >30 ppm PFOA group in 1993 and only 5 in 1995. Therefore, it is likely that there was not enough power to detect differences in either of the highest categories. The mean age of the employees in the highest exposure category was the lowest in both 1993 and 1995 (33.3 years and 38.2 years, respectively). Although not significantly different from the other categories, BMI was slightly higher in the highest PFOA category.

When the mean values of the various hormones were compared by exposure categories, there was a statistically significant ($p = .01$) elevation in prolactin in 1993 only for the 10 workers whose serum levels were between 10 and 30 ppm compared to the lower 2 exposure categories. In addition, TSH was significantly ($p = .002$) elevated in the same exposure category for 1995 only (mean blood serum level was 2.9 ppm). However, mean TSH levels for the other exposure categories, including the ≥ 30 ppm category, were all the same (1.7 ppm). In 1993, TSH was elevated only in this same exposure category, as well; however did not reach statistical significance ($p = .09$).

Estradiol levels in the >30 ppm group in both years were 10% higher than the other PFOA groups; however, the difference was not statistically significant ($p < 0.05$). These results were confounded by estradiol being highly correlated with BMI ($r = .41$, $p < .001$ in 1993, and $r = .30$, $p < .01$ in 1995). In 1995, all 5 employees with PFOA levels > 30 ppm had BMIs > 28, although this effect was not observed in 1993. The authors postulate that the study may not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (55 ppm PFOA in the CD rat). Only 3 employees in this study had PFOA serum levels this high. They also suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol.

The authors did not report a negative association between PFOA serum levels and testosterone. There were no statistically significant trends ($p < 0.05$) noted for PFOA and either bound or free testosterone. However, 17-HP, a precursor of testosterone, was highest in the >30 ppm PFOA group in both 1993 and 1995. In 1995, PFOA was significantly associated with 17-HP in

regression models adjusted for possible confounders. However, the authors state that this association was based on the results of one employee (data were not provided in the report). Free testosterone was highly correlated with age in both 1993 and 1995 ($r = -.48$, $p < .001$; $r = -.40$, $p < .001$, respectively).

There are several design issues that should be noted when evaluating the results of this study. First, although there were 2 study years (1993 and 1995), the populations were not independent. Sixty-eight employees participated in both years. Second, there were 31 fewer employees who participated in the study in 1995, thus reducing the power of the study. There were also very few employees in either year with serum PFOA levels greater than 10 ppm. Third, the cross-sectional design of the study does not allow for analysis of temporality of an association. Since the half-life of PFOA is at least 1 year, the authors suggest that it is possible that there may be some biological accommodation to the effects of PFOA. Fourth, only one sample was taken for each hormone for each of the study years. In order to get more accurate measurements for some of the hormones, pooled blood taken in a short time period should have been used for each participant. Fifth, some of the associations that were measured in this study were done based on the results of an earlier paper that linked PFOA with increased estradiol and decreased testosterone levels. However, total serum organic fluorine was measured in that study instead of PFOA, making it difficult to compare the results. Finally, there may have been some measurement error of some of the confounding variables.

3.1.3 Occupational Study on Episodes of Care

In order to gain additional insight into the effects of fluorochemical exposure on workers' health, an "episode of care" analysis was undertaken at the Decatur plant to screen for morbidity outcomes that may be associated with long-term, high exposure to fluorochemicals (Olsen et al., 2001g). An "episode of care" is a series of health care services provided from the start of a particular disease or condition until solution or resolution of that problem. Episodes of care were identified in employees' health claims records using Clinical Care Groups (CCG) software. All inpatient and outpatient visits to health care providers, procedures, ancillary services and prescription drugs used in the diagnosis, treatment, and management of over 400 diseases or conditions were tracked.

Episodes of care were analyzed for 652 chemical employees and 659 film plant employees who worked at the Decatur plant for at least 1 year between January 1, 1993 and December 31, 1998. Based on work history records, employees were placed into different comparison groups: Group A consisted of all film and chemical plant workers; Group B had employees who only worked in either the film or chemical plant; Group C consisted of employees who worked in jobs with high POSF exposures; and Group D had employees who worked in high exposures in the chemical plant for 10 years or more prior to the onset of the study. Film plant employees were considered to have little or no fluorochemical exposure, while chemical plant employees were assumed to have the highest exposures.

Ratios of observed to expected episodes of care were calculated for each plant. Expected numbers were based on 3M's employee population experience using indirect standardization techniques. A ratio of the chemical plant's observed to expected experience divided by the film plant's observed to expected experience was calculated to provide a relative risk ratio for each episode of care (RREpC). For each RREpC, 95% confidence intervals were calculated. Episodes of care that were of greatest interest were those which had been reported in animal or epidemiologic literature on PFOS and PFOA: liver and bladder cancer, endocrine disorders

involving the thyroid gland and lipid metabolism, disorders of the liver and biliary tract, and reproductive disorders.

The only increased risk of episodes for these conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers (RREpC = 8.2, 95% CI = 0.8 - 399). One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding should be noted because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study when there were only 2 exposure categories (chemical division employees and non-chemical division employees). The update of the study sub-divided the chemical plant employees and did not corroborate this finding when exposures were divided into definitely exposed and probably exposed employees.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. The limitations include: 1) episodes of care are reported, not disease incidence, 2) the data are difficult to interpret because a large RREpC

may not necessarily indicate high risk of incidence of disease, 3) many of the risk ratios for episodes of care had very wide confidence intervals, thereby providing unstable results, 4) the analysis was limited to 6 years, 5) the utilization of health care services may reflect local medical practice patterns, 6) individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications, 7) episodes of care may include the same individual several times, 8) not all employees were included in the database, such as those on long-term disability, 9) the analysis may be limited by the software used, which may misclassify episodes of care, 10) the software may assign 2 different diagnoses to the same episode, and 11) certain services, such as lab procedures may not have been reported in the database.

3.1.4 3M Medical Surveillance Studies

3.1.4.1 Antwerp and Decatur Plants–Cross-Sectional and Longitudinal Studies

A cross-sectional analysis of the data from the 2000 medical surveillance program at the Decatur and Antwerp plants was undertaken to determine if there were any associations between PFOA and hematology, clinical chemistries, and hormonal parameters of volunteer employees (Olsen et al., 2001e). The data were analyzed for all employees from both plant locations. Mean PFOA serum levels were 1.03 ppm for all male employees at the Antwerp plant and 1.90 ppm for all male employees at the Decatur plant. Male production employees at the Decatur plant had significantly higher ($p < .05$) mean serum levels (2.34 ppm) than those at the Antwerp plant (1.28 ppm). Non-production employees at both plants had mean levels below 1 ppm. PFOA serum levels were higher than the PFOS serum values at both plants, especially the Decatur plant where serum levels are higher overall. In addition, values for total organic fluorine were even higher than the PFOA levels.

Multivariable regression analyses were conducted to adjust for possible confounders that may affect the results of the clinical chemistry tests. The following variables were included: production job (yes or no), plant, age, BMI, cigarettes/day, drinks/day and years worked at the plant. A positive significant association was reported between PFOA and cholesterol ($p = .05$) and PFOA and triglycerides ($p = .002$). Age was also significant in both analyses. Alcohol consumed per day was significant in the cholesterol model, while BMI and cigarettes smoked per day was significant for triglycerides. When both PFOA and PFOS were included in the analyses, neither reached statistical significance in the cholesterol model, while PFOA remained significant ($p = .02$) in the triglycerides model. High-density lipoprotein (HDL) was negatively associated with PFOA ($p = .04$) and remained significant ($p = .04$) when both PFOA and PFOS were included in the model. A positive association ($p = .01$) between T3 and PFOA was also observed and remained statistically significant ($p = .05$) when PFOS was included in the model. BMI, cigarettes/day, alcohol/day were also significant in the model. None of the other clinical chemistry, thyroid or hematology measures were significantly associated with PFOA in the regression model.

A longitudinal analysis of the above data and previous medical surveillance results was performed to determine whether occupational exposure to fluorochemicals over time is related to changes in clinical chemistry and lipid results in employees of the Antwerp and Decatur facilities (Olsen et al., 2001f). The clinical chemistries included: cholesterol, HDL, triglycerides, alkaline phosphatase, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin. Medical surveillance data from 1995, 1997, and 2000 were analyzed using multivariable regression

analysis. The plants were analyzed using 3 subcohorts that included those who participated in 2 or more medical exams between 1995 and 2000. A total of 175 male employees voluntarily participated in the 2000 surveillance and at least one other. Only 41 employees were participants in all 3 surveillance periods.

When mean serum PFOA levels were compared by surveillance year, PFOA levels in the employees participating in medical surveillance at the Antwerp plant increased between 1994/95 and 1997 and then decreased slightly between 1997 and 2000. At the Decatur plant, PFOA serum levels decreased between 1994/95 and 1997 and then increased between 1997 and 2000. When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was a statistically significant positive association between PFOA and serum cholesterol ($p = .0008$) and triglycerides ($p = .0002$) over time. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees ($p = .005$) and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years ($p = .001$). However, the association between PFOA and triglycerides was also statistically significant ($p = .02$) for the subgroup in which employees participated in biomonitoring in 1994/95 and 2000. There was not a significant association between PFOA and triglycerides among Decatur workers. There were no significant associations between PFOA and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

There are several limitations to the 2000 cross-sectional and longitudinal studies including: 1) serum PFOA levels were significantly higher at the Decatur plant than at the Antwerp plant, 2) all participants were volunteers, 3) there were several consistent differences in clinical chemistry profiles and demographics between employees of the Decatur and Antwerp plants (Antwerp employees as compared to Decatur employees had lower PFOA serum levels, were younger, had lower BMIs, worked fewer years, had higher alcohol consumption, higher mean HDL and bilirubin values, lower mean triglyceride, alkaline phosphatase, GGT, AST, and ALT values, and mean thyroid hormone values tended to be higher), 4) PFOS and other perfluorinated chemicals are also present in these plants, 5) in the cross-sectional study, plant populations cannot be compared because they were placed into quartiles based on PFOS serum distributions only which were different for each subgroup and not applicable to PFOA, 6) only one measurement at a certain point in time was collected for each clinical chemistry test, and 7) PFOA serum levels overall have been increasing over time in these employees. In addition, in the longitudinal study only a small number of employees participated in all 3 sampling periods (24%), different labs and analytical techniques for PFOA were used each year, and female employees could not be analyzed because of the small number of participants.

3.1.4.2 Cottage Grove Plant–Cross-sectional Studies of Clinical Chemistries and CCK

A voluntary medical surveillance program was offered to employees of the Cottage Grove, Minnesota plant in 1993, 1995, and 1997 ($n = 111$, 80 and 74 employees, respectively) (Olsen et al., 1998b, 2000). The clinical chemistry parameters (cholesterol, hepatic enzymes, and lipoprotein levels) used in the longitudinal and cross-sectional studies of the Antwerp and Decatur plants were also used in this study. In addition, in 1997 only, cholecystokinin-33 (CCK) was also measured at the Cottage Grove plant. CCK levels were observed because certain research has suggested that pancreas acinar cell adenomas seen in rats exposed to PFOA may be the result of increased CCK levels (Obourn et al., 1997).

Only male employees involved in PFOA production were included in the study. Sixty-eight employees were common to the 1993 and 1995 sampling periods, 21 were common between

1995 and 1997, and 17 participated in all three surveillance years. Cottage Grove has the highest serum PFOA levels of the 3 plants studied.

Employees' serum PFOA levels were stratified into 3 categories (<1, 1- <10, and ≥10 ppm), chosen to provide a greater number of employees in the ≥ 10 ppm category. As employees' mean serum PFOA levels increased, no statistically significant ($p < 0.05$) abnormal liver function tests, hypolipidemia, or cholestasis were observed in any of the sampling years. Multivariable regression analyses controlling for potential confounders (age, alcohol consumption, BMI, and cigarettes smoked) yielded similar results. The authors also reported that renal function, blood glucose, and hematology measures were not associated with serum PFOA levels; however, these data were not provided in the paper.

The mean CCK value reported for the 1997 sample was 28.5 pg/ml (range 8.8 - 86.7 pg/ml). The means in the 2 serum categories < 10 ppm were at least 50% higher than in the ≥ 10 ppm category. A statistically significant ($p = .03$) negative association between mean CCK levels and the 3 PFOA serum categories was observed. A scatter plot of the natural log of CCK and PFOA shows that all but 2 CCK values are within the assay's reference range of 0 - 80 pg/ml. Both of these employees (CCK values of 80.5 and 86.7 pg/ml) had serum PFOA levels less than 10 ppm (0.6 and 5.6 ppm, respectively). A multiple regression model of the natural log of CCK and serum PFOA levels continued to display a negative association after adjusting for potential confounders.

The cross-sectional design is a limitation of this study. Only 17 subjects were common to all 3 sampling years. In addition, the medical surveillance program is a voluntary one. The participation rate of eligible production employees decreased from approximately 70% in 1993 to 50% in 1997. Also, the laboratory reference range changed substantially for ALT in 1997. Finally, different analytical methods were used to measure serum PFOA. Serum PFOA was determined by electrospray high-performance liquid chromatography/mass spectrometry in 1997, but by thermospray in 1993 and 1995.

3M used data collected in their 2000 medical surveillance program to determine whether serum PFOA levels greater than 5 ppm were associated with changes in hepatic, lipid and thyroid function in workers (Olsen, et al., 2003f). Clinical chemistries, including thyroid function tests, were performed for 131 male and 17 female workers at the plant. Serum samples were extracted and quantitatively analyzed for PFOA using HPLC/EMSS. All of the samples were above the lower limit of quantitation (LLOQ) (see Table 24 in Section 4.1.1 for biomonitoring data).

Fifteen percent (n = 20) of male employees had serum concentrations that exceeded 5 ppm, and none of the female employees were above 5 ppm. Number of years worked in the Chemical Division of the plant were not correlated with PFOA serum measurements, but were correlated with specific production areas. When the male employees were separated into 3 groups based on serum PFOA levels (< 1 ppm, 1- 4.9 ppm, and >5 ppm), there were no statistically significant ($p < .05$) differences in mean lipid and hepatic test results or in thyroid hormone levels, between the 3 groups both before and after adjusting for potential confounders (eg. BMI, smoking status, and alcohol consumption). The elimination of employees receiving cholesterol-reducing drugs (n = 9) from the analysis did not alter these findings. Similar to findings at the Decatur and Antwerp plants, triglyceride levels were higher in employees with the highest PFOA serum concentrations, although not statistically significant ($p < .05$). In simple linear regression analyses, a weak negative association between T4 and serum PFOA concentrations was reported ($p = .07$). However, all of the serum PFOA concentrations were within the T4 reference range,

the statistical association explained minimal variation in the model ($r^2 = .03$), there was no increase in serum TSH or T3 levels, and no negative association between free T4 levels and PFOA. No statistically significant ($p < 0.05$) associations between PFOA and clinical chemistries or thyroid test results for the small group of female employees was reported.

An earlier medical surveillance study on workers who were employed in the 1980's was conducted at the Cottage Grove plant; however, total serum fluorine was measured instead of PFOA (Gilliland and Mandel, 1996). Based on animal studies that reported that animals exposed to PFOA develop hepatomegaly and alterations in lipid metabolism, a cross-sectional, occupational study was performed to determine if similar effects are present in workers exposed to PFOA. In a PFOA production facility, 115 workers were studied to determine whether serum PFOA affected their cholesterol, lipoproteins, and hepatic enzymes. Forty-eight workers who were exposed to PFOA from 1985-1989 were included in the study (96% participation rate). Sixty-five employees who either volunteered or were asked to participate, were included in the unexposed group. These employees were assumed to have little or no PFOA exposure based on their job description. However, when serum levels were analyzed, it was noted that this group of workers had PFOA levels much greater than the general population. Therefore, instead of job categories, total serum fluorine was used to classify workers into exposure groups.

Total serum fluorine was used as a surrogate measure for PFOA. Serum PFOA was not measured, due to the cost of analyzing the samples. Blood samples were analyzed for total serum fluorine, serum glutamyl oxaloacetic transaminase (SGOT or AST), serum glutamyl pyruvic transaminase (SGPT or ALT), GGT, cholesterol, low-density lipoproteins (LDL), and HDL. All of the participants were placed into five categories of total serum fluorine levels: <1 ppm, 1-3 ppm, >3 - 10 ppm, >10 - 15 ppm, and > 15 ppm. The range of the serum fluorine values was 0 to 26 ppm (mean 3.3 ppm). Approximately half of the workers fell into the > 1 - 3 ppm category, while 23 had serum levels < 1 ppm and 11 had levels > 10 ppm.

There were no significant differences between exposure categories when analyzed using univariate analyses for cholesterol, LDL, and HDL. In the multivariate analysis, there was not a significant association between total serum fluorine and cholesterol or LDL after adjusting for alcohol consumption, age, BMI, and cigarette smoking. There were no statistically significant differences among the exposure categories of total serum fluorine for AST, ALT and GGT. However, increases in AST and ALT occurred with increasing total serum fluorine levels in obese workers (BMI = 35 kg/m^2). This result was not observed when PFOA was measured directly in serum of workers in 1993, 1995, or 1997 surveillance data of employees of the Cottage Grove plant (Olsen et al., 2000).

Since PFOA was not measured directly and there is no exposure information provided on the employees (e.g. length of employment/exposure), the results of the study provide limited information. The authors state that no adverse clinical outcomes related to PFOA exposure have been observed in these employees; however, it is not clear that there has been follow-up of former employees. In addition, the range of results reported for the liver enzymes were fairly wide for many of the exposure categories, indicating variability in the results. Given that only one sample was taken from each employee, this is not surprising. It would be much more helpful to have several samples taken over time to ensure their reliability. It also would have been interesting to compare the results of the workers who were known to be exposed to PFOA to those who were originally thought not to be exposed to see if there were any differences among the employees in these groups. There were more of the "unexposed" employees (n = 65) participating in the study than those who worked in PFOA production (n = 48).

3.2 Metabolism and Pharmacokinetics

3.2.1 Metabolism and Pharmacokinetic Studies in Humans

Little is known about the metabolism and pharmacokinetics of PFOA in humans. One report notes the presence of PFOA in the cord blood of some pregnant workers suggesting that PFOA can cross the placenta (U.S. EPA, 2003). In addition, there are limited data on the half-life of PFOA in humans. With the exception of a 1980 study in which total organic fluorine in blood serum was measured in one worker, no other data were available until June 2000 (Ubel et al., 1980). A half-life study on 27 retirees from the Decatur and Cottage Grove 3M plants was undertaken, in which serum samples were drawn every 6 months over a 5-year period. Two interim reports describing the results thus far have been submitted (Burris et al., 2000, 2002). The first interim report suggested a median serum half-life of PFOA of 344 days, with a range of 109 to 1308 days. The two highest half-life calculations were for the 2 female retirees who participated in this study (654 and 1308 days).

There were several limitations to this first analysis including: 1) the limited data available and the range of serum PFOA levels measured; 2) serum was analyzed after each collection period with only one measurement per time period on different days using slightly different analytical techniques; and 3) the reference material purity was not determined until after the first 3 samples had been analyzed.

An effort was made to minimize experimental error, including systematic and random error in the analytical method. Serum samples were collected from 9 of the original 27 subjects over 4 time periods spanning 180 days, measured in triplicate with all time points from each subject analyzed in the same analytical run. This would allow for statistical evaluation of the precision of the measurement and assure that all systematic error inherent in the assay equally affected each sample used for half-life determination. Single serum measurements were made on samples of the remaining 18 retirees, but were not included by the investigators in the analysis because triplicate analyses of all time points were not conducted.

Of the 9 retirees included in this analysis, there were 7 males and 2 females, all from the Decatur plant. The average age of the retirees was 61 years, the mean number of years worked at Decatur was 27.7 years, and the average number of months retired from the plant at study initiation was 18.9. The average BMI of this group was 27.9 (range 22.5-33, SD = 3.6). The mean PFOA value at study initiation was 0.72 ppm (range 0.06 – 1.84 ppm, SD = 0.64).

The mean serum half-life for PFOA was 4.37 years (range 1.50 – 13.49 years, SD = 3.53). Only 1 employee had a half-life value that exceeded 4.3 years. The 2 females had values of 3.1 and 3.9 years. Age, BMI, number of years worked or years since retirement were not significant predictors of serum half-lives in multivariable regression analyses.

This analysis has attempted to reduce experimental error in the determination of a half-life for PFOA. However, two issues should be noted. First, the effect of continual non-occupational, low-level exposure on the half-life is unknown. Second, systematic error of the analytical method could be as high as +/- 20% and still satisfy the data quality criteria.

3.2.2 Metabolism and Pharmacokinetic Studies in Non-Human Primates

Noker and Gorman (2003) administered a single intravenous (i.v.) dose of 10 mg/kg potassium

PFOA to 3 male and 3 female cynomolgus monkeys. The animals were approximately 3-4 years of age at the start of the study. Each monkey was examined shortly after dosing for clinical signs of toxicity; all animals were observed twice daily for signs of mortality/moribundity. Additional clinical observations were performed on the days blood was collected. Each animal was weighed on days 1, 4, 7, 14, 21 and 28. Urine and feces were collected on days 0, 1, 2, 7, 14, 21, and 28. Blood was collected at 0, 0.5, 2, 4, 8 and 24 hours and on days 2, 4, 7, 11, 14, 21, 28, 57, 79, 87, and 123. Serum and urine samples were analyzed by HPLC/MS/MS.

No deaths that could be attributed to administration of the test article occurred during the study. One male was euthanized on day 79 because he had developed repeated episodes of self-mutilation. These episodes do not appear to be related to PFOA administration. No adverse clinical signs were noted in any of the other monkeys during the course of the study. Body weights of the treated animals did not change between days 1 and 28.

At 0.5 hours after dosing, serum concentration in males and females were similar, and ranged from 91,130 to 96,660 ng/ml (ppb) in males and from 88,940 to 96,400 ng/ml in females. Serum concentrations declined slowly, and the levels decreased faster in two of the male monkeys than in the third male monkey and all three female monkeys. By day 123, PFOA concentrations were at or slightly above the limit of quantitation (20 ng/ml) in the two surviving males and between 885 and 4701 ng/ml in the three females.

The pharmacokinetic parameters that were calculated from the serum concentrations of PFOA are presented in Table 1. The estimated values for half-life and total body clearance indicated that two of the three male monkeys eliminated PFOA at a faster rate than did the female monkeys. The volume of distribution of PFOA at steady state was similar for both sexes. Male #2052 appeared to behave more like the females in his pharmacokinetic parameters than either of the other two males in the study. One explanation is the stress of the experiment caused this monkey to release high levels of cortisol that could have affected carbohydrate, protein, and/or lipid metabolism, caused a shift in electrolyte and water balance, or increased plasma proteins. This animal was also observed to have periods of severe self-mutilation and euthanized for this reason on day 79.

Table 1

Pharmacokinetic Parameters Calculated from Serum Concentrations of PFOA in Cynomolgus Monkeys

	Males					Females				
Parameter	2052	2054	2211	Mean	S.D.	2058	2059	2061	Mean	S.D.
Cmax (µg/mL)[a]	101	98.4	91.6	97.0	4.9	105	93.0	103	100	6.0
$t_{½}$ (day)[b]	35.3	13.7	13.6	20.9	12.5	26.8	41.7	29.3	32.6	8.0
$AUC_{0\text{-last}}$ (µg•day/mL)[c]	1999	569	633	1067	808	3083	2314	1056	2151	1023
$AUC_{0\text{-infinity}}$ (µg•day/mL)[d]	2501	571	634	1235	1097	3224	2560	1094	2293	1090
Cl (mL/day/kg)[e]	4.0	17.5	15.8	12.4	7.7	3.1	3.9	9.1	5.4	3.3
Vd_{ss} (mL/kg)[f]	192	168	184	181	12	133	190	270	198	69

a Maximum concentration in serum
b Half-life of the terminal elimination phase
c Area under the serum concentration versus time curve calculated from 0 to the last time point
d Area under the serum concentration versus time curve calculated from 0 to infinity
e Total body clearance
f Volume of distribution at steady state

There were no obvious sex differences in the urinary excretion of PFOA. The rate of urinary excretion by the monkeys was slow. Less than 20% of the administered dose was excreted in the urine of the male and female monkeys within the first 48 hours after dosing, and as much as 0.1 - 1% of the administered dose was excreted in the urine on day 28.

3.2.3 Metabolism and Pharmacokinetic Studies in Adult Rats

3.2.3.1 Absorption Studies

Studies in rats have shown that PFOA is absorbed following oral, inhalation, and dermal exposures.

3.2.3.1.1 Oral Exposure

Gibson and Johnson (1979) administered a single dose of 11.0 mg/kg ^{14}C-PFOA by gavage to groups of 3 male 10-week old CD rats. Twenty-four hours after administration, at least 93% of the total carbon-14 was absorbed; the elimination half-life of carbon-14 from the plasma was 4.8 days.

Ophaug and Singer (1980) administered 2 ml of an aqueous solution of 2 mg PFOA to female Holtzman rats. Ionic fluoride, nonionic fluorine, and total fluorine were measured. Within 4.5 hr, 37% of the total fluorine in the administered dose was recovered in the urine. The quantity of nonionic fluorine recovered in the urine increased to 61%, 76% and 89% at 8, 24 and 96 hr, respectively, after administration. Within 4.5 hr, serum from treated rats had a nonionic fluorine level of 13.6 ppm. The nonionic fluorine level in the serum decreased to 11.2 ppm at 8 hr, 0.35 ppm at 24 hr, and 0.08 ppm at 96 hr. Despite the large decrease in nonionic fluorine in the serum, the ionic fluoride level was only 0.03 ppm and remained at that level throughout the experiment. Prior to administration of PFOA, the ionic and nonionic fluorine background levels in serum were 0.032 and 0.07 ppm, respectively. The authors concluded that PFOA is rapidly absorbed from the gastrointestinal tract and then rapidly cleared from the serum.

3.2.3.1.2 Inhalation Exposure

Hinderliter (2003) measured the serum concentrations of PFOA following single and repeated inhalation exposures in Sprague-Dawley rats. For the single exposure study, male and female rats (3/sex/group) were exposed to a single nose-only exposure of an aerosol of 0, 1, 10, or 25 mg/m^3 PFOA. Preliminary range-finding studies demonstrated that aerosol sizes were 1.8 - 2.0 μm mass median aerodynamic diameter (MMAD) with geometric standard deviations ranging from 1.9 - 2.1 μm. Blood samples were collected pre-exposure, at 0.5, 1, 3 and 6 hours during exposure, and at 1, 3, 6, 12, 18 and 24 hours after exposure. Plasma was analyzed by LC-MS. PFOA plasma concentrations were proportional to the inhalation exposure concentrations. The male C_{max} values were approximately 2-3 times higher than the female C_{max}. The female C_{max} occurred approximately one hour after the exposure period, while the male C_{max} occurred from the end of the exposure period up to six hours after exposure. In females, the elimination of PFOA was rapid at all exposure levels, and by 12 hours after exposure the plasma levels had dropped below the analytical limit of quantitation (0.1 μg/ml). In males, the plasma elimination was much slower, and at 24 hours after exposure, the plasma concentrations were approximately 90% of the peak concentrations at all exposure levels.

In the repeated dose study, Hinderliter (2003) exposed male and female rats (5/sex/group) to the same aerosol concentrations of PFOA for 6 hrs/day, 5 days/week for 3 weeks. Blood was collected immediately before and after the daily exposure period three days per week. The aerosol sizes were 1.3 - 1.9 μm MMAD with geometric standard deviations of 1.5 - 2.1. PFOA plasma concentrations were proportional to the inhalation exposure concentrations, and repeated exposures produced little plasma carryover in females, but significant carryover in males. Male rats reached steady state plasma levels by three weeks with plasma concentrations of 8, 21, and 36 μg/ml for the 1, 10 and 25 mg/m^3 groups, respectively. In females, the post-exposure plasma levels were 1, 2, and 4 μg/ml for the 1, 10, and 25 mg/m^3 groups, respectively. When measured immediately before the daily exposure, plasma levels had returned to baseline in females.

3.2.3.1.3 Dermal Exposure

No specific dermal absorption studies have been conducted in rats. However, Kennedy (1985) treated rats dermally with a total of 10 applications of APFO at doses of 0, 20, 200 or 2,000 mg/kg. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner.

3.2.3.2 Serum Pharmacokinetic Parameters in Adult Rats

Serum pharmacokinetic parameters of PFOA have been evaluated in adult Sprague-Dawley rats following gavage administration, and in Wistar rats following i.v. administration.

3.2.3.2.1 Oral and Intravenous Exposure in Sprague-Dawley Rats

Kemper (2003) examined the plasma concentration profile of PFOA following gavage and intravenous administration in sexually mature Sprague-Dawley rats. Male and female rats (4/sex/group) were administered single doses of PFOA by gavage at dose rates of 0.1, 1, 5, and 25 mg/kg PFOA, and intravenously at a dose rate of 1 mg/kg PFOA. After dosing, plasma was collected for 22 days in males and 5 days in females. Plasma concentration vs. time data were then analyzed by non-compartmental pharmacokinetic methods (Tables 2 and 3). Comparison of AUC for the oral and intravenous 1 mg/kg doses indicated that oral bioavailability of PFOA was approximately 100%. Plasma elimination curves were linear with respect to time in male rats at all dose levels, while elimination kinetics were biphasic in females at the 5 mg/kg and 25 mg/kg dose levels. In males, plasma elimination half-lives were independent of dose level and ranged from approximately 138 hours to 202 hours. In females, terminal elimination half-lives ranged from approximately 2.8 hours at the lowest dose to approximately 16 hours at the high dose. To further characterize plasma elimination kinetics, particularly in male rats, animals were given oral PFOA at a rate of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (24 hours and 2016 hours in females and males, respectively). Estimated plasma elimination half-lives in this experiment were approximately 277 hours in males and 3.4 hours in females (Tables 2 and 3).

Table 2
Pharmacokinetic Parameters in Male Sprague-Dawley Rats
Following Administration of PFOA by Gavage (Mean (SD))

Parameter	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg i.v.	0.1 mg/kg extended time
T_{max} (hr)	10.25 (6.45)	9.00 (3.83)	15.0 (10.5)	7.5 (6.2)	NA	5.5 (7.0)
C_{max} (µg/mL)	0.598 (0.127)	8.431 (1.161)	44.75 (6.14)	160.0 (12.0)	NA	1.08 (0.42)
Lambda z (1/hr)	0.004 (0.001)	0.005 (0.001)	0.0041 (0.0007)	0.0046 (0.0012)	0.004 (0.000)	0.0026 (0.0007)
$T_{1/2}$ (hr)	201.774 (37.489)	138.343 (31.972)	174.19 (28.92)	157.47 (38.39)	185.584 (19.558)	277.10 (56.62)
AUC_{INF} (hr·µg/mL)	123.224 (35.476)	1194.463 (215.578)	6733.70 (1392.83)	25155.61 (7276.96)	1249.817 (113.167)	206.38 (59.03)
AUC_{INF}/D (hr·µg/mL/mg/kg)	1096.811 (310.491)	1176.009 (206.316)	1221.89 (250.28)	942.65 (284.67)	1123.384 (100.488)	2111.28 (586.77)
Cl_p (mL/kg·hr)	0.962 (0.240)	0.871 (0.158)	0.85 (0.21)	1.13 (0.31)	0.896 (0.082)	0.51 (0.17)

Table 3
Pharmacokinetic Parameters in Female Sprague-Dawley Rats
Following Administration of PFOA by Gavage (Mean (SD))

Parameter	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg i.v.	0.1 mg/kg extended time
T_{max} (hr)	0.56 (0.31)	1.13 (0.63)	1.50 (0.58)	1.25 (0.87)	NA	1.25 (0.50)
C_{max} (µg/mL)	0.67 (0.07)	4.782 (1.149)	20.36 (1.58)	132.6 (46.0)	NA	0.52 (0.08)
Lambda z (1/hr)	0.231 (0.066)	0.213 (0.053)	0.15 (0.02)	0.059 (0.037)	0.250 (0.047)	0.22 (0.07)
$T_{1/2}$ (hr)	3.206 (0.905)	3.457 (1.111)	4.60 (0.64)	16.22 (9.90)	2.844 (0.514)	3.44 (1.26)
AUC_{INF} (hr·µg/mL)	3.584 (0.666)	39.072 (10.172)	114.90 (11.23)	795.76 (187.51)	33.998 (7.601)	3.34 (0.32)
AUC_{INF}/D (hr·µg/mL/mg/kg)	31.721 (5.880)	38.635 (10.093)	20.78 (2.01)	29.54 (6.92)	30.747 (6.759)	34.39 (3.29)
Cl_p (mL/kg·hr)	32.359 (6.025)	27.286 (7.159)	48.48 (4.86)	35.06 (.88)	34.040 (9.230)	29.30 (3.06)

3.2.3.2.2 Intravenous Exposure in Wistar Rats

Kudo et al. (2002) examined the plasma concentration profile of PFOA following i.v. administration in 9-week old Wistar rats. Male and female rats were injected intraveneously with 48.63 µmol/kg body weight PFOA, and blood samples were collected at 5 min., 1, 6, 12 hrs, and 1, 2, 3, 4, 6, 8, 10 and 12 days after injection. A two-compartment open model was used to estimate serum pharmacokinetic parameters (Table 4). The half-life of PFOA in male rats was found to be 70 times longer (5.7 days versus 1.9 hours) than in females. In female rats, there appears to be biphasic elimination of PFOA; the fast phase occurs with a half-life of approximately 1.9 hours while the slow phase occurs with a half life of approximately 24 hours. The total clearance of PFOA in female rats was 44 times higher than that in male rats.

Table 4
Pharmacokinetic Parameters of PFOA in Wistar Rats after i.v. Administration

Parameters	Male	Female
Total clearance (ml/min per kg)	0.035±0.010	1.551 ± 0.559*
$T_{1/2}$ (day)	5.68±0.99	0.08 ± 0.03**
Volume of distribution (ml/kg)	345.6±57.3	211.2+ 28.2*

Values are means ± S.D. for three to four rats. Differences were statistically significant between

male and female rats (*, $P<0.01$: **, $P<0.001$).

3.2.3.3 Distribution Studies in Adult Rats

The distribution of PFOA has been examined in tissues of adult rats following administration by gavage and by i.v. and intraperitoneal (i.p.) injection. PFOA distributes primarily to the liver, plasma, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue.

3.2.3.3.1 Oral Exposure

Kemper (2003) examined the distribution and clearance of PFOA in tissues of male and female Sprague-Dawley rats following administration by gavage. Rats were administered 1, 5, and 25 mg/kg ^{14}C-PFOA by oral gavage. Tissue concentrations were determined at the time of maximum plasma concentration (T_{max}) and at the time plasma concentration had fallen to one half the maximum ($T_{max/2}$). Values for T_{max} and $T_{max/2}$ for male and female rats were determined from pharmacokinetic experiments. $T_{max/2}$ was calculated as $T_{max} + T_{1/2}$. For cases in which biphasic elimination was evident, the rapid phase $T_{1/2}$ was used for calculation of $T_{max/2}$. Tissues from male rats were collected at 10.5 hours (T_{max}) and 171 hours ($T_{max/2}$) after dosing. Tissues from female rats were collected at 1.25 hours (T_{max}) and 4 hours ($T_{max/2}$) after dosing. The results are summarized in Tables 5 and 6 for males and females, respectively. Liver, kidney and blood were the primary tissues for distribution of ^{14}C-PFOA. In males, the fraction of the dose found in liver increased from T_{max} to $T_{max/2}$, but remained constant or decreased in other tissues. In females, the fraction of the dose present in all tissues remained constant or decreased between T_{max} and $T_{max/2}$. Liver-to-blood concentration ratios for ^{14}C at T_{max} in males were greater than 1, and increased between T_{max} and $T_{max/2}$. Kidney-to-blood concentration ratios at T_{max} in females were approximately 2 at all dose levels and remained relatively constant between T_{max} and $T_{max/2}$.

Table 5
Distribution of PFOA in Male Sprague-Dawley Rats after Oral Exposure
(Percent of dose recovered at T_{max} and $T_{max/2}$ in tissues)

	1 mg/kg	1 mg/kg	5 mg/kg	5 mg/kg	25 mg/kg	25 mg/kg
	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$
prostate	0.083±0.039	0.030±0.002	0.071±0.045	0.057±0.020	0.067±0.018	0.028±0.012
skin[a]	14.77±2.135	6.061±0.274	15.565±0.899	7.233±0.430	13.836±0.969	5.419±0.237
blood[a]	22.148±0.692	8.232±1.218	24.919±1.942	11.140±0.624	22.905±1.177	7.904±1.032
brain	0.071±0.018	0.022±0.002	0.051±0.021	0.023±0.008	0.063±0.007	0.019±0.002
fat[a]	2.281±0.467	0.593±0.136	2.815±0.225	0.916±0.205	2.153±0.430	0.628±0.110
heart	0.451±0.119	0.195±0.024	0.443±0.037	0.252±0.030	0.461±0.053	0.164±0.032
lungs	0.74±0.147	0.341±0.043	0.593±0.376	0.344±0.194	0.863±0.103	0.303±0.057
spleen	0.086±0.011	0.045±0.006	0.096±0.017	0.060±0.007	0.106±0.015	0.042±0.005
liver	21.708±5.627	32.627±3.601	18.750±2.434	25.231±1.289	17.528±0.900	20.145±3.098
kidney	1.949±0.402	1.14±0.215	2.170±0.354	1.212±0.115	2.293±0.286	1.003±0.122
G.I. tract	2.930±0.929	0.980±0.300	2.508±0.713	1.052±0.202	2.784±0.608	0.808±0.189
G.I. contents	2.083±0.625	0.239±0.025	2.632±0.934	0.270±0.028	4.186±1.349	0.210±0.084
thyroid	0.008±0.005	0.004±0.003	0.011±0.006	0.004±0.002	0.009±0.002	0.005±0.001
thymus	0.085±0.008	0.051±0.018	0.085±0.012	0.053±0.003	0.120±0.025	0.045±0.010
testes	0.755±0.079	0.356±0.037	0.693±0.180	0.372±0.062	0.623±0.098	0.224±0.031
adrenals	0.019±0.004	0.010±0.001	0.022±0.004	0.009±0.001	0.026±0.004	0.009±0.003
muscle[a]	12.025±0.648	4.984±0.745	13.565±0.576	6.429±0.648	12.855±0.841	4.253±0.358
bone[a]	3.273±0.538	1.120±0.094	3.047±0.544	1.375±0.169	3.062±0.438	0.906±0.100
Total[b]	85.465±6.426	57.026±3.379	88.033±1.420	56.031±1.025	83.937±3.680	42.112±4.740

[a] Percent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.
[b] Totals are calculated from individual animal data.

Table 6
Distribution of PFOA in Female Sprague-Dawley Rats after Oral Exposure
(Percent of dose recovered at T_{max} and $T_{max/2}$ in tissues)

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$
skin[a]	0.434±0.162	0.403±0.096	0.624±0.142	0.307±0.121	0.380±0.166	0.415±0.175
blood[a]	5.740±1.507	4.438±1.625	8.089±2.080	5.411±1.466	7.158±2.232	6.407±1.406
brain	0.037±0.009	0.047±0.008	0.066±0.019	0.045±0.010	0.058±0.008	0.058±0.018
fat[a]	0.134±0.032	0.164±0.079	0.220±0.111	0.110±0.069	0.147±0.053	0.148±0.065
heart	0.198±0.079	0.253±0.055	0.388±0.057	0.236±0.051	0.317±0.035	0.287±0.069
lungs	0.454±0.148	0.546±0.082	0.827±0.102	0.570±0.179	0.678±0.067	0.775±0.204
spleen	0.063±0.027	0.058±0.006	0.101±0.021	0.060±0.012	0.091±0.007	0.070±0.002
liver	7.060±1.266	6.817±1.537	11.190±2.192	7.176±0.982	10.538±1.723	9.080±0.895
kidney	3.288±0.948	2.769±0.784	4.293±0.771	2.685±0.736	5.867±0.946	4.749±0.393
G.I. tract	10.699±9.066	8.462±6.519	7.142±2.594	8.255±8.967	6.923±1.846	3.547±1.306
G.I. contents	21.956±13.48	3.891±2.395	2.896±2.305	5.601±6.165	2.491±1.548	1.121±1.010
thyroid	0.010±0.003	0.016±0.021	0.008±0.002	0.006±0.002	0.009±0.003	0.007±0.002
thymus	0.052±0.017	0.058±0.024	0.105±0.030	0.068±0.021	0.091±0.032	0.077±0.020
ovaries	0.047±0.019	0.048±0.006	0.071±0.012	0.041±0.012	0.071±0.012	0.070±0.012
adrenals	0.014±0.005	0.018±0.004	0.026±0.005	0.015±0.004	0.031±0.005	0.021±0.001
muscle[a]	0.170±0.051	0.258±0.089	0.325±0.010	0.229±0.031	0.441±0.116	0.304±0.099
uterus	0.243±0.091	0.374±0.247	0.354±0.046	0.247±0.068	0.358±0.124	0.365±0.029
bone[a]	0.101±0.017	0.153±0.052	0.174±0.057	0.142±0.078	0.157±0.072	0.181±0.090
Total[b]	50.698±16.48	28.772±10.98	36.897±3.187	31.201±12.63	35.803±2.554	27.680±2.569

[a] Percent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.
[b] Totals are calculated from individual animal data.

3.2.3.3.2 Intravenous Exposure

Gibson and Johnson (1980) examined the tissue distribution in 10-week old male CD rats following a single i.v. dose of 13.1 mg/kg ^{14}C-PFOA. The animals were sacrificed 36 days after dosing. The results are presented in Table 7. PFOA distributed mainly to the liver, plasma and kidney.

Table 7
Distribution of PFOA in Male CD Rats After i.v. Exposure

Tissue	Mean ± SD[a]
Liver	7.97 ± 4.02
Plasma	3.19 ± 1.72
Kidney	2.33 ± 1.13
Lung	0.79 ± 0.51
Red Blood Cells	0.77 ± 0.45
Skin	0.40 ± 0.28
Spleen	0.33 ± 0.19
Bone marrow	0.33 ± 0.26
Subcutaneous Fat	0.28 ± 0.18
Muscle	0.20 ± 0.09
Brain	0.11 ± 0.04
Abdominal Fat	< 0.12

a - Total ^{14}C concentration expressed as µg equivalents of ^{14}C-PFOA.

3.2.3.3.3 Intraperitoneal Exposure

Ylinen et al. (1990) studied the distribution and elimination of PFOA in a limited sample of tissues following a single i.p. dose of 50 mg/kg in 10 week old Wistar rats (20/sex). Samples were collected from 2 animals per sex for analysis of PFOA at 12, 24 - 168 (in 24 hour intervals), 224 and 336 hours after dosing. The concentration of PFOA in the serum and tissues was determined with capillary gas chromatography equipped with a flame ionization detector (FID). A mass spectrometer was used in the selected ion monitoring mode when the PFOA concentration was below the quantitation limit of the FID (1 µg/ml). The half-life of PFOA was estimated from the linear regression of time and concentration of PFOA in a semilogarithmic plot. No PFOA was detected in the adipose tissue. In both sexes at 12 hours after administration, the highest concentration of PFOA was found in the serum, followed by the liver, kidney, spleen and brain. In the females, the concentration of PFOA in the serum, liver and kidney decreased in a discontinuous fashion indicating distinct phases. The half-life of PFOA in

the serum, liver, kidney and spleen is presented in Table 8; the values are generally much lower in females than in males.

Table 8
Mean Half-life (hours) of PFOA in Wistar Rats After i.p. Exposure

Tissue	Males	Females
Serum	105	24
Liver	210	60
Kidney	130	145
Spleen	170	73

Vanden Heuvel et al. (1991b) examined the distribution of PFOA in the serum and a limited number of tissues following administration of 9.4 μmol/kg ^{14}C-PFOA by i.p. injection to 6-week old male and female Harlan Sprague-Dawley rats. At various time-points for 28 days following treatment, four rats per sex were sacrificed; blood was collected by cardiac puncture, and tissues were removed and frozen. ^{14}C-PFOA-derived radioactivity was quantitated using a Liquid Scintillation Analyzer. Assuming first order kinetics, tissue elimination rates were calculated as $T_{½} = \ln 2/k_e$. The distribution and elimination of PFOA-derived ^{14}C in selected tissues is summarized in Table 9. In the male rats, 21% of the administered dose was present in the liver, and the next highest concentrations were found in the plasma and kidney. Far lower PFOA concentrations were found in the heart, testis, fat, and gastrocnemius muscle. In females, the highest concentrations of PFOA were found in the plasma followed by the kidney, liver and ovaries. In males, PFOA was eliminated from the liver at a slower rate than the other tissues; the $T_{½}$ for liver was 11 days compared to 8-9 days in most extrahepatic tissues. The rates of elimination were much faster in the female rats than in the male rats.

The high concentration of PFOA in the male liver was further examined using a liver perfusion technique. Liver was infused with 0.08 μmol ^{14}C-PFOA/min over a 48 min period for a total of 3.84 umol ^{14}C. Approximately 11% of the cumulative dose of ^{14}C-PFOA infused was extracted by the liver in a first pass. At 2 min, the cumulative percent of PFOA extracted by the liver was 33%; that was substantially greater than the 11% cumulative dose of ^{14}C that was extracted after 48 min indicating that first-pass hepatic uptake of PFOA may be saturable.

Table 9
Distribution and Elimination of PFOA in Selected Tissues in Harlan Sprague-Dawley Rat after i.p. Exposure[a]

Tissue	% Dose[a]				Elimination Rate[b]		Half-life	
	Male (2 hrs)	Female (2 hrs)	Male (24 hrs)	Female (24 hrs)	Male (day^{-1})	Female ($hour^{-1}$)	Male (day)	Female (hour)
Liver	2.03 (0.10)	1.53 (0.25)	2.08 (0.14)	0.06 (0.02)	0.062 (0.005)	0.185 (0.011)	11.3	3.8
Plasma	1.99 (0.03)	2.39 (0.44)	1.63 (0.11)	0.02	0.077 (0.006)	0.242 (0.007)	9.0	2.9
Kidney	0.95 (0.08)	2.00 (0.38)	0.74 (0.03)	0.06 (0.02)	0.074 (0.005)	0.214 (0.012)	9.4	3.2
Heart	0.42 (0.02)	ND	0.39 (0.03)	ND	0.066 (0.006)	ND	10.4	ND
Gastrocnemius	0.26 (0.01)	ND	0.21 (0.01)	ND	0.076 (0.007)	ND	9.2	ND
Fat[c]	0.32 (0.02)	ND	0.27 (0.04)	ND	0.087 (0.008)	ND	8.0	ND
Testis/ Ovary	0.33 (0.01)	0.53 (0.10)	0.27 (0.03)	0.05 (0.01)	0.078 (0.006)	ND	9.0	ND

ND - Not determined
a- Values are percent dose of PFOA-derived ^{14}C per gram tissue; mean (SEM); n=4; values with no SEM indicate that SEM < 0.01
b- Mean (SEM); n=32 for males and n=16 for females
c-Epididymal fat pad

3.2.3.4 Metabolism Studies in Adult Rats

Several studies have examined metabolism of PFOA. However, no studies show clear evidence of metabolism. Ophaug and Singer (1980) found no change in ionic fluoride level in the serum or urine following oral administration of PFOA to female Holtzman rats. Ylinen et al. (1989) found no evidence of phase II metabolism of PFOA following a single intraperitoneal PFOA dose (50 mg/kg) in male and female Wistar rats.

Vanden Heuvel et al. (1991b) investigated the metabolism of PFOA in Harlan Sprague-Dawley rats administered ^{14}C-PFOA (9.4 μmol/kg, i.p.). Pooled daily urine samples (0-4 days post-treatment) and bile extracts analyzed by HPLC contained a single radioactive peak eluting identically to the parent compound. Tissues were taken from rats treated 4, 14, and 28 days after treatment to determine the presence of PFOA-containing lipid conjugates. Only the parent compound was present in rat tissues; no PFOA-containing hybrid lipids were detected. Fluoride concentrations in plasma and urine before and after PFOA treatment were unchanged, indicating that PFOA does not undergo defluorination.

3.2.3.5 Elimination Studies in Adult Rats

In adult rats, there is evidence of enterohepatic circulation of PFOA. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats. There are gender differences in the elimination of PFOA in rats. The rapid excretion of PFOA by female rats is due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to change the rate of elimination in rats.

3.2.3.5.1 Enterohepatic Circulation

Johnson et al. (1984) investigated the effect of feeding cholestyramine to rats on the elimination of APFO. Since APFO exists as an anion at physiologic pH, it would be expected to complex with cholestyramine. Ten male Charles River CD rats, 12 weeks of age, were given a single i.v. injection of 13 mg/kg ^{14}C-APFO. Five rats were given 4% cholestyramine in feed. Urine and feces samples were collected at intervals for 14 days, at which time the animals were sacrificed and liver samples were collected. At 14 days post dose, the mean percentage of PFOA eliminated in the feces of cholestyramine-treated rats was 9.8-fold the mean percentage eliminated in the feces of rats that did not receive cholestyramine. Excretion in urine was 41% of the administered dose for cholestyramine treated rats and 67% for rats that did not receive cholestyramine. ^{14}C in the liver equaled 4% or 12.1 ± 2.1 μg eq/g in cholestyramine treated rats and 8 % or 22.3 ± 6.2 μg eq/g in rats that did not receive cholestryamine. In plasma, the levels were 5.1 ± 1.7 μg eq/ml in cholestyramine treated rats and 14.7±6.8 μg eq/ml in rats that did not receive cholestyramine. In red blood cells, the levels were 1.8 ± 0.7 μg eq/ml in cholestryamine treated rats and 4.2 ± 2.4 μg eq/ml in rats that did receive cholestryamine. The high concentration of ^{14}C-APFO in the liver at 2 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of ^{14}C nearly 10-fold suggests that there is enterohepatic circulation of PFOA.

3.2.3.5.2 General Elimination Studies

3.2.3.5.2.1 Oral Exposure

Kemper (2003) investigated the elimination of PFOA in male and female Sprague-Dawley rats (4 rats/sex/group) administered a single dose of ^{14}C-PFOA by oral gavage at dose levels of 1, 5, and 25 mg/kg. Urine and feces were collected for 28 days in males and 7 days in females. Urine was the primary route of excretion of ^{14}C in both sexes, accounting for 43-62% of the administered dose in males and 76-84% of the administered dose in females. Cumulative recovery of ^{14}C in the urine increased gradually over the 28 days in male rats, but was essentially complete in female rats within the first 72 hours. Fecal excretion of ^{14}C accounted for 6-14% of the dose in males and 2-6% of the dose in females. Pilot experiments demonstrated that ^{14}C was not eliminated as either $^{14}CO_2$ or volatile organic compounds in ^{14}C-PFOA-treated rats. Pretreatment of rats with 1 mg/kg-day PFOA for 14 days had little or no effect on the excretion of a challenge dose of 1 mg/kg ^{14}C-PFOA.

3.2.3.5.2.2 Intravenous Exposure

Gibson and Johnson (1980) examined the excretion of total ^{14}C in male and female CD rats after a single i.v. dose of ^{14}C-PFOA. The mean dose for females was 16.7 mg/kg while that for males was 13.1 mg/kg. Female rats excreted essentially all of the administered dose via the urine in the

24 hours after treatment. During the same time period, male rats excreted only 20% of the total dose. Male rats excreted 83% of the total dose via the urine and 5.4% via the feces by 36 days post dose. No radioactivity was detected in tissues of female rats at 17 days post dose; 2.8% of the total dose was detected in the liver of male rats and 1.1% in the plasma at 36 days post dose with lower levels equaling < 0.5% of the total dose in other organs.

3.2.3.5.3 Elimination Studies in the Pregnant Rat

Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination of ^{14}C after oral administration of a single dose of ^{14}C-APFO (Gibson and Johnson, 1983). At 8 or 9 days after conception, four pregnant CD rats and two nonpregnant female CD rats were given a mean dose of 15 mg/kg ^{14}C-APFO. Individual urine samples were collected at 12, 24, 36, and 48 hours post dose and analyzed for ^{14}C content. Essentially all of the ^{14}C was eliminated via the urine within 24 hours for both groups of rats.

3.2.3.5.4 Studies on the Mechanism of the Gender Difference in Elimination in Adult Rats

Several studies have been conducted to elucidate the cause of the gender difference in rats in the elimination of PFOA. Hanhijarvi et al. (1982) conducted a series of studies to examine the effect of probenecid, which inhibits the renal active secretion system for organic acids, on the elimination of PFOA in male and female Holtzman rats. In the first study, 4 male and 6 female Holtzman rats were administered 2 mg of nonionic fluorine as PFOA by gavage. Seven female rats were administered 2 ml distilled water as controls. The animals were then placed in metabolism cages and urine was collected until the animals were sacrificed at 24 hr by cardiac puncture. Serum was collected. Ionic fluoride and total fluorine content of serum and urine were determined, and nonionic fluorine was calculated as the difference. Twenty-four hours after oral administration of PFOA, female rats had excreted 76 ± 2.7% of the dose in the urine and had a mean serum nonionic fluorine level of 0.35 ± 0.11 μg/ml, while male rats had excreted only 9.2 ± 3.5% of the dose and had a mean serum nonionic fluorine level of 44.0 ± 1.7 μg/ml. A mean of 97.5±0.25% of the PFOA was bound in the plasma of both male and female rats.

In the second study, Hanhijarvi et al. (1982) examined the effect of probenecid on the clearance of PFOA and inulin. Holtzman rats were anesthetized and the femoral artery was cannulated for continuous infusion of 5% mannitol in isotonic saline, while the femoral artery was cannulated for drawing blood samples. The urinary bladder was also cannulated for serial collections of urine. When the urine and serum collections for the clearance study were complete, 65-68 mg/kg probenecid was administered by i.p. injection and after 20 - 30 minutes, additional 10 minute clearance tests were performed. Administration of probenecid reduced the PFOA/inulin clearance ratio in females from 14.5 to 0.46. PFOA clearance was reduced from 5.8 to 0.11 ml/min/100g. Net PFOA excretion was reduced from 4.6 μg/min/100g to 0.13 μg/min/100g. In male rats, however, the PFOA/inulin clearance ratio and the net excretion of PFOA were virtually unaffected by probenecid. In the males, PFOA clearance was 0.17 ml/min/100g, the PFOA/inulin clearance ratio was 0.22, and net PFOA excretion was 0.17 μg/min/mg.

Finally, Hanhijarvi et al. (1982) examined the cumulative excretion of PFOA over a 7-hour period. Holtzman rats were dosed i.v. with a mixture of 10%-20% radiolabeled-PFOA and 80-90% unlabeled PFOA. Mannitol (5%) was infused and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65-68 mg/kg by i.p. injection at least 30 min prior to the administration of PFOA. Female rats excreted 76% of the administered dose of PFOA, while males excreted only 7.8% of the administered dose over a 7-

hr period. Probenecid administration modified the cumulative excretion curve for males only slightly. However, in females probenecid markedly reduced PFOA elimination to 11.8%. The authors concluded that the female rat possesses an active secretory mechanism which rapidly eliminates PFOA from the body.

Ylinen et al. (1989) studied the urinary excretion of PFOA in male Wistar rats after castration and estradiol administration. Twenty male rats were castrated at 28 days of age and were used in tests of PFOA excretion 5 weeks later. Ten castrated and 10 intact males were given 500 μg/kg estradiol valerate by s.c. injection every second day for 14 days before administration of PFOA. PFOA was administered as a single i.p. injection at 50 mg/kg. Urine was collected in metabolism cages for 96 hr after PFOA administration. Blood samples were collected by cardiac puncture. Six female rats were also included in the experiment. Castration and administration of estradiol to the male rats had a significant stimulatory effect on the urinary excretion of PFOA. During the first 24 hours, female rats excreted 72 ± 5% of the administered dose of PFOA, whereas the intact males excreted only 9 ± 4%. After the estradiol treatment, both the intact and castrated males excreted PFOA in amounts similar to females, 61 ± 19% and 68 ± 14%, respectively. The castrated males without estradiol treatment excreted 50 ± 13% of the administered dose of PFOA in the urine. This was faster than the intact males but less than the females and the estrogen treated males. At the end of the test, the concentration of PFOA in the serum of intact males was 17- 40 times higher than the concentration PFOA in the serum of other groups. There were no statistically significant differences in the serum concentrations between the other groups. PFOA was similarly bound by the proteins in the serum of males and females.

Vanden Heuvel et al. (1992a) investigated whether androgens or estrogens are involved in the marked sex-differences in the urinary excretion of PFOA. Castrated Harlan Sprague-Dawley male rats were given 9.4 μmol/kg ^{14}C-PFOA by i.p. injection. Castration increased the elimination of PFOA in the urine (36% of the dose was eliminated in 4 days versus 16% in controls), suggesting that a factor produced by the testis is responsible for the slow elimination of PFOA in male rats. Castration plus 17B-estradiol had no further effect on PFOA elimination whereas castration plus testosterone replacement at the physiological level reduced PFOA elimination to the same level as rats with intact testis. Thus, in male rats, testosterone exerts an inhibitory effect on renal excretion of PFOA. In female rats, neither ovariectomy or ovariectomy plus testosterone affected the urinary excretion of PFOA, demonstrating that the inhibitory effect of testosterone on PFOA renal excretion is a male-specific response. Probenecid, which inhibits the renal transport system, decreased the high rate of PFOA renal excretion in castrated males but had no effect on male rats with intact testis.

Kudo et al. (2002) examined the role of sex hormones on the renal clearance (CL_R) of PFOA and the renal mRNA levels of specific organic anion transporters in male and female Wistar rats. Castration of male rats caused a 14-fold increase in CL_R of PFOA. The elevated PFOA CL_R in castrated males was reduced by treating them with testosterone. Treatment of male rats with estradiol increased the CL_R of PFOA. In female rats, ovariectomy caused a significant increase in CL_R of PFOA, which was reduced by estradiol treatment. Treatments of female rats with testosterone reduced the CL_R of PFOA. Treatment with probenecid, a known inhibitor of organic anion transporters, markedly reduced the CL_R of PFOA in male rats, castrated male rats, and female rats. To identify the transporter molecules that are responsible for PFOA transport in the rat kidney, renal mRNA levels of specific organic anion transporters were determined in male and female rats under various hormonal states and compared with the CL_R of PFOA. The level of OAT2 mRNA in male rats was only 13% that in female rats. Castration or estradiol treatment increased the level of OAT2 mRNA whereas treatment of castrated male rats with

testosterone reduced it. Ovariectomy of female rats significantly increased the level of OAT3 mRNA. Multiple regression analysis of the data suggested that organic anion transporter 2 (OAT2) and OAT3 are responsible for urinary elimination of PFOA in the rat.

3.2.4 Metabolism and Pharmacokinetic Studies in Immature Rats

No studies have been conducted to specifically examine the absorption, metabolism or elimination of PFOA in the developing rat. However, recent studies have been conducted to examine the concentrations of PFOA in the developing Sprague-Dawley rat, and to determine when the gender difference in elimination of PFOA becomes apparent. In addition, several studies have examined the serum and tissue distribution of PFOA in newly weaned Wistar rats. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. During lactation and immediately after weaning, the elimination of PFOA is similar in males and females. In the male rat between 4-5 weeks of age, the factor(s) responsible for the gender difference develop, and the rats assume the adult male elimination profile. In addition, distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

3.2.4.1 PFOA Levels During Pregnancy and Lactation

Mylchreest (2003) examined PFOA levels during gestation and lactation. Pregnant Sprague-Dawley rats were dosed with 0, 3, 10 or 30 mg/kg-day APFO during days 4-10, 4-15, or 4-21 of gestation, or from gestation day 4 to lactation day 21. Clinical observations and body weights were recorded daily. On gestation days 10, 15, and 21, 5 rats/group/time point were sacrificed and the number, location and type of implantation sites were recorded. Embryos were collected on day 10, and placentas, amniotic fluid, and embryos/fetuses were collected on days 15 and 21. Maternal blood samples were collected at 2 hours $\pm$ 30 minutes post-dose. The remaining 5 rats per group were allowed to deliver. On lactation days 0, 3, 7, 14, and 21, the pups were counted, weighed (sexes separate), and examined for abnormal appearance and behavior. Randomly selected pups were sacrificed and blood samples were collected. On lactation days 3, 7, 14, and 21, the dams were anesthetized and milk and blood samples were collected; dams were removed from their litters 1-2 hours prior to collection. Plasma, milk, amniotic fluid extract, and tissue homogenates (placenta, embryo, and fetus) supernatants were analyzed for PFOA concentrations by HPLC-MS.

All dams survived and there were no clinical signs of toxicity. In the 30 mg/kg-day group, mean body weight gain was approximately 10% lower than the control group during gestation, and mean body weights were approximately 4% lower than controls throughout gestation and lactation. The number of implantation sites, resorptions, and live fetuses were comparable among groups on days 10, 15, and 21 of gestation. One dam in the 3 mg/kg-day group and two dams in the 30 mg/kg-day group delivered small litters (litter size of 3-6 pups as compared to 12-19 pups/litter in the control group); however, given the small sample size the biological significance of this finding is unclear. There were no clinical signs of toxicity in the pups, and pup survival and pup body weights were comparable among groups.

Maternal PFOA levels during gestation and lactation are presented in Table 10. Maternal plasma levels at 2 hrs post-dosing (approximately the time of peak blood levels following a gavage dose) were fairly similar during the course of the study with a mean level of 11.2, 26.8, and 66.6 µg/ml in the 3, 10, and 30 mg/kg-day groups, respectively; PFOA levels in the control group were below the limit of quantitation (0.05 µg/ml). The concentration of PFOA in the milk was

also fairly similar throughout lactation and was approximately 1/10th of the PFOA levels in the plasma; the mean values were 1.1, 2.8, and 6.2 μg/ml in the 3, 10, and 30 mg/kg-day groups, respectively.

Table 10
Maternal PFOA Levels (μg/ml) During Gestation and Lactation[a]

Exposure Period	Sample Time	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
GD 4 - GD 10	GD 10 plasma	8.53 ± 1.06	23.32 ± 2.15	70.49 ± 8.94
GD 4 - GD 15	GD 15 plasma	15.92 ± 12.96	29.40 ± 14.19	79.55 ± 3.11
GD 4 - GD 21	GD 21 plasma	14.04 ± 2.27	34.20 ± 6.68	76.36 ± 14.76
GD 4 - LD 3	LD 3 - plasma - milk	11.01 ± 2.11 1.07 ± 0.26	22.47 ± 2.74 2.03 ± 0.33	54.39 ± 17.86 4.97 ± 1.20
GD 4 - LD 7	LD 7 - plasma - milk	10.09 ± 2.90 0.94 ± 0.22	25.83 ± 2.07 2.74 ± 0.91	66.91 ± 11.82 5.76 ±1.26
GD 4 - LD 14	LD 14 - plasma - milk	9.69 ± 0.92 1.15 ± 0.06	23.79 ± 2,81 3.45 ± 1.18	54.65 ± 11.63 6.45 ± 1.38
GD 4 - LD 21	LD 21 - plasma - milk	9.04 ± 1.01 1.13 ± 0.08	28.84 ± 5.15 3.07 ± 0.51	64.13 ± 1.45 7.48 ± 1.63
NA	Average plasma Average milk	11.19 ± 2.76 1.07 ± 0.09	26.84 ± 4.21 2.82 ± 0.60	66.64 ± 9.80 6.16 ± 1.06

a- mean ± SD; samples were from 5 dams/group/time point and were collected 2 hrs post-dosing

PFOA levels in the placenta, amniotic fluid, embryo, fetus, and pup plasma are presented in Table 11. The levels of PFOA in the placenta on gestation day 21 were approximately twice the levels observed on gestation day 15, and the levels of PFOA in the amniotic fluid were approximately four times higher on day 21 than on day 15. The concentration of PFOA in the embryo/fetus was highest in the day 10 embryo and lowest in the day 15 embryo; PFOA levels in the day 21 fetus were intermediate. The concentration of PFOA in the plasma of the day 21 fetus were approximately half the levels observed in the maternal plasma; the mean values were 5.9, 14.5, and 33.1 μg/ml in the 3, 10, and 30 mg/kg-day groups, respectively. Pup plasma levels decreased until lactation day 7, and were thereafter similar to the levels observed in the milk.

Table 11
PFOA Concentrations (μg/ml) During Gestation and Lactation in Sprague-Dawley Rats[a]

Exposure Period	Tissue	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
GD 4 - GD 10	GD 10 - embryo	1.40 ± 0.30	3.33 ± 0.81	12.49 ± 3.50
GD 4 - GD 15	GD 15 - placenta - amniotic fluid - embryo	2.22 ± 1.79 0.60 ± 0.69 0.24 ± 0.19	5.10 ± 1.70 0.70 ± 0.15 0.53 ± 0.18	13.22 ± 1.03 1.70 ± 0.91 1.24 ± 0.22
GD 4 - GD 21	GD 21 - placenta - amniotic fluid - fetus - fetal plasma	3.55 ± 0.57 1.50 ± 0.32 1.27 ± 0.26 5.88 ± 0.69	9.37 ± 1.76 3.76 ± 0.81 2.61 ± 0.37 14.48 ± 1.51	24.37 ± 4.13 8.13 ± 0.86 8.77 ± 2.36 33.11 ± 4.64
GD 4 - LD 3	LD 3 - pup plasma	2.89 ± 0.70	5.94 ± 1.44	11.96 ± 1.66
GD 4 - LD 7	LD 7 - pup plasma	0.65 ± 0.20	2.77 ± 0.58	4.92 ± 1.28
GD 4 - LD 14	LD 14 - pup plasma	0.77 ± 0.10	2.22 ± 0.38	4.91 ± 1.12
GD 4 - LD 21	LD 21 - pup plasma	1.28 ± 0.72	3.25 ± 0.52	7.36 ± 2.17

a - mean ± SD; samples were pooled by litter and were collected 2 hrs post-dosing

3.2.4.2 PFOA Levels in the Postweaning Rat

Han (2003) examined the relationship between age and plasma PFOA concentrations in the postweaning Sprague-Dawley rat. Four to eight week old rats (10/sex/time period) were administered a single dose of 10 mg/kg-day APFO by gavage. Blood samples were collected 24 hours after dosing and the plasma concentration of PFOA was measured by HPLC-MS. At four weeks of age, the concentration of plasma PFOA was approximately 2.7 times higher in males than in the females (Table 12). In females, the concentration of plasma PFOA decreased by 2.7 fold between 4 and 5 weeks of age, and thereafter remained fairly steady. In males, the concentration of plasma PFOA increased by 5.4 fold between 4 and 5 weeks of age, and thereafter remained fairly steady. Between 5 and 8 weeks of age, the PFOA plasma concentrations were 34.7 - 65.1 fold higher in males than in females of the same age. Thus, it appears that the elimination of PFOA is similar among males and females until week 4, and between weeks 4 and 5 the maturation of the factor(s) responsible for the gender difference in elimination of PFOA occurs in the male rat.

Table 12
Plasma PFOA Concentrations (μg/ml) in Postweaning Sprague-Dawley Rats[a]

Age (weeks)	Males	Females
4	7.32 ± 1.01	2.68 ± 0.64
5	39.24 ± 3.89	1.13 ± 0.46
6	43.19 ± 3.79	1.18 ± 0.52
7	37.12 ± 4.07	0.57 ± 0.29
8	38.55 ± 5.44	0.81 ± 0.27

a - mean ± SD; samples from 10 animals/sex/group

3.2.4.3 Serum and Tissue Distribution in Immature Wistar Rats Following Oral Exposure

Ylinen et al. (1990) administered newly weaned Wistar rats (18/sex/group) doses of 3, 10, and 30 mg/kg-day PFOA by gavage for 28 days. At necropsy, serum was collected as well as the brain, liver, kidney, lung, spleen, ovary, testis, and adipose tissue. The concentration of PFOA in the serum and tissues was determined with capillary gas chromatography equipped with a flame ionization detector (FID). A mass spectrometer was used in the selected ion monitoring mode when the PFOA concentration was below the quantitation limit of the FID (1 μg/ml). The concentration of PFOA in the serum and tissues following 28 days of administration is presented in Table 13. PFOA was not detected in the adipose tissue. The concentrations of PFOA in the serum and tissues were much higher in males than in females. In the males, the levels of PFOA in the serum and tissues were generally lower in the 30 mg/kg-day group than in the 10 mg/kg-day group due to increased urinary elimination in the 30 mg/kg-day group.

Table 13
Tissue Distribution of PFOA in Wistar Rats after 28 Days of Treatment

Tissue	Males[a]			Females[a]		
	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
Serum	48.6±10.3	87.27±20.09	51.65±1.47	2.4[b]	12.47±4.07	13.92±6.06
Liver	39.9±7.25	51.71±11.18	49.77±10.76	1.81±0.49	3.45±1.36	6.64±2.64
Kidney	1.55±0.71	40.56±14.94	39.81±17.67	0.06±0.02	7.36±3.19	12.54±8.24
Spleen	4.75±1.66	7.59±3.5	4.1±1.57	0.15±0.04	0.38±0.17	1.59±0.49
Lung	2.95±0.54	22.58±4.59	23.71±5.42	0.24[b]	0.22±0.15	0.75±0.26
Brain	0.398±0.144	1.464±0.211	0.71±0.32	< LOQ[c]	0.029±0.019	0.044±0.018
Ovary				< LOQ	0.41±0.27	1.16±0.58
Testis	6.24 ± 2.04	9.35 ± 4.02	7.22 ± 3.17			

a- N = 6, Mean ± SD, µg/ml
b - N=3, no SD
c - below the limit of quantitation

Hanhijarvi et al. (1987) administered PFOA by gavage to 48 newly-weaned Wistar rats (6/sex/group) at 0, 3, 10, and 30 mg/kg-day for 28 consecutive days and determined the serum levels of PFOA. At the end of the study, blood was collected via cardiac puncture; PFOA levels were determined by gas chromatography. At each dose level, the mean PFOA concentrations in the plasma of the male rats were significantly higher than those of the female rats. The mean plasma PFOA concentrations for the male rats were 48.6 ± 26.5 µg/ml, 83.1 ± 24.7 µg/ml, and 53.4 ± 11.2 µg/ml, respectively for the 3, 10 and 30 mg/kg-day dose levels. The corresponding figures for female rats were 2.43 ± 5.96 µg/ml, 11.3 ± 8.59 µg/ml, and 9.06 ± 8.80 µg/ml respectively, for the 3, 10 and 30 mg/kg-day dose levels.

3.2.5 Comparative Studies of Protein Binding in Humans, Non-Human Primates, and Rats

It has been suggested that PFOA circulates in the body by noncovalently binding to plasma proteins. Several studies have investigated the binding of PFOA to plasma proteins of rats, humans or monkeys to gain understanding of its absorption, distribution and elimination, and species and gender differences.

Protein binding of plasma from cynomolgus monkeys, rats, and humans was tested with PFOA (SRI, 2003). The results are summarized in Table 14. Most PFOA binds to human serum albumin compared to other protein components of human plasma at physiological concentrations. Rat, human, and monkey plasma all bind PFOA at 97- 100% at tested concentrations ranging from 1-500 ppm.

Table 14
% Protein Binding to Rat, Human and Monkey Plasma

Nominal Concentration (ppm)	Rat	Monkey	Human
1	~100	~100	~100
10	99.5	99.8	99.9
100	98.6	99.8	99.9
250	97.6	99.8	99.6
500	97.3	99.5	99.4

% Binding values reported as "~100" reflect a nonquantifiable amount of test article in the plasma water BQL<6.25 ng/ml

Han et al. (2003) investigated the binding of PFOA to rat and human plasma proteins in vitro. Rats treated in vivo and then sacrificed showed no gender difference in the binding of PFOA to serum, though the persistence of PFOA in vivo is much greater in male than female rats. The authors conclude that there is no correlation between the PFOA persistence and binding of the PFOA to rat serum. The primary PFOA binding protein in plasma was serum albumin. However, the method used (ligand blotting) would not theoretically allow the identification of low abundance proteins with high affinity for PFOA. Further investigation of purified rodent and human serum albumin binding using labeled ^{19}F NMR allowed the calculation of disassociation constants for PFOA binding to rodent and human serum albumin. No significant differences between binding to the two proteins was detected (Table 15).

Table 15
Dissociation Constants (K_d) of Binding between PFOA and Rodent Serum Albumin (RSA) and Human Serum Albumin (HSA) and the Number of PFOA Binding Sites (n) on RSA and HSA

Parameter	Method	RSA	HSA
K_d (mM)	NMR[a]	0.29 ± 0.10[c]	
K_d (mM)	micro-SEC[b]	0.36 ± 0.08[c]	0.38 ± 0.04
n	micro-SEC[b]	7.8 ± 1.5	7.2 ± 1.3

[a]Average of the two K_d values (0.31 ± 0.15 and 0.27 ± 0.05 mM) obtained by NMR. [b]Values were obtained from three independent experiments and their standard deviations are shown. [c]On the basis of the result of unpaired t-test at 95% confidence interval, the difference of K_d values determined by NMR and micro-SEC is statistically insignificant.

3.2.6 Metabolism and Pharmacokinetic Studies in Other Test Species

There is limited information on the metabolism and pharmacokinetics of PFOA in mice, rabbits and dogs. No specific pharmacokinetic studies have been conducted in mice. However, toxicology studies in mice indicate that PFOA is absorbed, and furthermore, there does not

appear to be a gender difference in elimination. For example, Sohlenius et al. (1992) exposed male and female C57B1/6 mice to dietary levels of 0.02% PFOA for one week. There was a significant decrease in mean body weight and a significant increase in absolute liver weight; the response was similar in male and female mice.

In rabbits, there is no information available on the metabolism or elimination of PFOA by any route of exposure. No specific studies of the absorption of PFOA have been conducted following oral or inhalation exposure. However, there is evidence that PFOA is absorbed following dermal exposure. O'Malley and Ebbins (1981) treated male and female New Zealand White rabbits dermally with doses of 100, 1,000 and 2,000 mg/kg APFO for 14 days. Mortality was 100% (4/4) in the 2,000 mg/kg group, 75% (3/4) in the 1,000 mg/kg group and 0% (0/4) in the 100 mg/kg group. Similarly, Kennedy (1985) treated rabbits dermally with a total of 10 applications of APFO at doses of 0, 20, 200 or 2,000 mg/kg. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner.

In addition, limited information is available on the serum and liver distribution of PFOA in rabbits following i.v. administration. Johnson (1995a) administered individual female rabbits intravenous doses of 0, 4, 16, 24 and 40 mg/kg tetrabutyl ammonium salt of PFOA. The animal given 40 mg/kg died within 5 minutes of treatment. All other animals appeared normal throughout the study. Serum samples were analyzed for total organic fluorine at 2, 4, 6, 8, 12, 24, and 48 hours post dose. At 2 hrs, serum organic fluorine levels in the rabbits that received 0, 4, 16, and 24 mg/kg were 1.25 μg/ml, 4.09 μg/ml, 14.9 μg/ml, and 41.0 μg/ml, respectively. There was a rapid decrease of total organic fluorine in the serum with time; it was non-detectable at 48 hr. The biological half-life was on the order of 4 hours. The total organic fluorine levels in whole liver at 48 hr post dose for the rabbits that received 0 mg/kg, 4 mg/kg, 16 mg/kg, and 24 mg/kg were 20 μg, 43 μg, 66 μg, and 54 μg, respectively.

There is no information on the specific absorption, metabolism or distribution of PFOA in dogs by any route of exposure. One study examined the elimination of PFOA in dogs following i.v. administration. Hanhijarvi et al. (1988) administered beagle dogs (3/sex) an i.v. injection of 30 mg/kg of PFOA followed by continuous infusion with 5% mannitol. Urine and blood were collected at 10 minute intervals for 60 min. Probenecid was then administered by i.v. injection, and urine and blood samples were collected as before. Renal clearance of PFOA was calculated for the before and after probenecid injection periods. Four additional dogs (2/sex) were given 30 mg/kg of PFOA by i.v. injection. These dogs were kept in metabolism cages, and blood samples were collected intermittently for 30 days. The renal clearance rate was approximately 0.03 ml/min/kg. Probenecid significantly reduced the PFOA clearance rate in both sexes, indicating an active secretion mechanism for PFOA. The plasma half-life of PFOA was 473 hr before probenecid administration and 541 hr after in male dogs, and 202 hr before probenecid and 305 hr after in the female dogs.

3.3 Acute Toxicity Studies in Animals

Dean and Jessup (1978) reported an oral LD50 of 680 mg/kg and 430 mg/kg for male and female CD rats, respectively. Glaza (1997) reported an oral LD50 of greater than 500 mg/kg in male Sprague-Dawley rats and between 250 and 500 mg/kg in females. Gabriel (1976d) reported an oral LD50 of less than 1000 mg/kg for male and female Sherman-Wistar rats. Rusch (1979) reported no mortality in male or female Sprague-Dawley rats following inhalation exposure to 18.6 mg/L for one hour. The dermal LD50 in New Zealand White rabbits was determined to be greater than 2000 mg/kg (Glaza, 1995).

APFO is an ocular irritant in rabbits when the compound is not washed from the eyes (Gabriel, 1976b, 1976e), but is not an irritant in rabbits when washed from the eye (Gabriel, 1976a). Markoe (1983) found APFO to be a skin irritant in rabbits, while Gabriel (1976c) did not.

3.4 Mutagenicity Studies

APFO was tested twice (Lawlor, 1995; 1996) for its ability to induce mutation in the *Salmonella – E. coli*/mammalian-microsome reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen at one dose level in S. typhimurium TA1537 when tested without metabolic activation was not reproducible. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation. APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli, 1996c; NOTOX, 2000). Sadhu (2002) recently reported that APFO did not induce gene mutation when tested with or without metabolic activation in the K-1 line of Chinese hamster ovary (CHO) cells in culture.

Murli (1996b) tested APFO twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy (Murli, 1996b).

APFO was tested in a cell transformation and cytotoxicity assay conducted in C_3H $10T_{½}$ mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation potential. There was no evidence of transformation at any of the dose levels tested in either the colony or foci assay methods (Garry & Nelson, 1981).

APFO was tested twice in the mouse micronucleus assay. APFO did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay (Murli, 1996a).

3.5 Repeat Dose Studies in Animals

3.5.1 Subchronic Studies in Non-Human Primates

Goldenthal (1978b) administered rhesus monkeys (2/sex/group) doses of 0, 3, 10, 30 or 100 mg/kg-day APFO by gavage for 90 days. Animals were observed twice daily and body weights were recorded weekly. Blood and urine samples were collected once during the control period, and at 1 and 3 months of the study for hematology, clinical chemistry and urinalysis. Organs and tissues from animals that were sacrificed at the end of the study and from animals that died during the treatment period were weighed, examined for gross pathology and processed for histopathology.

All monkeys in the 100 mg/kg-day group died during the study. The first death occurred during week 2; all animals were dead by week 5. Signs and symptoms which first appeared during week 1 included anorexia, frothy emesis which was sometimes brown in color, pale face and gums, swollen face and eyes, slight to severe decreased activity, prostration and body trembling. Three monkeys from the 30 mg/kg-day group died during the study; one male died during week

7 and the two females died during weeks 12 and 13. Beginning in week 4, all four animals showed slight to moderate and sometimes-severe decreased activity. One monkey had emesis and ataxia, swollen face, eyes and vulva, as well as pallor of the face and gums. Beginning in week 6, two monkeys had black stools and one monkey had slight to moderate dehydration and ptosis of the eyelids. No monkeys in the 3 or 10 mg/kg-day groups died during the study. One monkey in the 10 mg/kg-day group was anorexic during week 4, had a pale and swollen face in week 7 and had black stools for several days in week 12. Animals in the 3 mg/kg-day group occasionally had soft stools or moderate to marked diarrhea; frothy emesis was also occasionally noted in this group.

Changes in body weight were similar to the controls for animals from the 3 and 10 mg/kg-day groups. Monkeys from the 30 and 100 mg/kg-day groups lost body weight after week 1. At the end of the study, this loss was statistically significant for the one surviving male in the 30 mg/kg-day group (2.30 kg vs 3.78 kg for the control). The results of the urinalysis, and hematological and clinical chemistry analyses were comparable for the control and the 3 and 10 mg/kg-day groups at one and three months.

At necropsy, the following changes in absolute and relative organ weight changes were noted: absolute and relative weight of the hearts in females from the 10 mg/kg-day group were significantly decreased; absolute brain weight of females from this same group were also significantly decreased and relative group mean weight of the pituitary in males from the 3 mg/kg-day group was significantly increased. The biological significance of these weight changes is difficult to assess, as they were not accompanied by morphologic changes.

In animals that died before the end of the study, one male and two females from the 30 mg/kg-day group and all animals from the 100 mg/kg-day group had marked diffuse lipid depletion in the adrenal glands. All males and females from the 30 and 100 mg/kg-day groups also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. One female from the 30 mg/kg-day group and all animals in the 100 mg/kg-day group had moderate atrophy of the lymphoid follicles in the lymph nodes.

Only one male in the 30 mg/kg-day group survived until terminal sacrifice, and this male also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. No treatment related lesions were seen in the organs of animals from the 3 and 10 mg/kg-day groups.

The levels of PFOA in the serum and liver of these animals are presented below in Table 16. Individual values are presented so there are double entries for most dose levels.

Table 16
Levels of PFOA in the Serum and Liver of Surviving Rhesus Monkeys

Dose	Serum (μg/ml)		Liver (μg/ml)		Liver total (μg)	
	Male	Female	Male	Female	Male	Female
0	ND	1	0.05	0.07	3	5
3	53	65	3	7	250	350
3	48	50	ND	ND	ND	ND
10	45	79	9	ND	600	ND
10	71	71	ND	10	ND	750
30	145	Dead	61	Dead	4000	Dead

ND - Not Determined

Under the conditions of this study the LOAEL is 3 mg/kg-day and no NOAEL was established because effects were observed at the lowest dose.

Thomford (2001a,b) conducted a range-finding and a 6-month toxicity study in male cynomolgus monkeys. In the range-finding study, Thomford (2001a) administered male cynomolgus monkeys an oral capsule containing 0, 2, or 20 mg/kg-day APFO for 4 weeks. There were 3 monkeys in the 2 and 20 mg/kg-day groups and one monkey in the control group. The monkeys weighed 2.1 to 3.6 kg at the start of treatment. Animals were observed twice daily for mortality and moribundity and were examined at least once daily for signs of poor health or abnormal behavior. Body weights were recorded weekly and food consumption was assessed qualitatively. The monkeys were fasted overnight and blood samples were collected one week prior to the start of the study and on day 30 for clinical hematology and clinical chemistry analyses, and hormone and PFOA level. Blood for clinical chemistry was also collected from each animal on day 2 (approximately 24 hours after the first dose). Samples were analyzed for estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin.

At scheduled necropsy, samples of the right lateral lobe of the liver were collected from each animal and analyzed for palmitoyl CoA oxidase activity. Representative samples of liver, right and left testes, and pancreas were collected from each animal for cell proliferation evaluation using proliferation cell nuclear antigen. Bile was collected from each animal for bile acid determination. A sample of liver was collected from each animal for PFOA concentration analysis. The adrenals, liver, pancreas, spleen, and testes from each animal were examined microscopically, and the remaining tissues were preserved for possible future examination.

All animals survived to scheduled sacrifice. There were no clinical signs of toxicity in the treated groups and there was no effect on body weight. Low or no food consumption was observed for one animal given 20 mg/kg-day. There were no effects on estradiol, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin. Estrone levels were notably lower for males given 2 and 20 mg/kg-day APFO. There was no evidence of

peroxisome proliferation or cell proliferation in the liver, testes or pancreas of treated monkeys. No adverse effects were noted in either gross or clinical pathology studies.

In the 26-week study, male cynomolgus monkeys were administered APFO by oral capsule at doses of 0, 3, 10 or 30 mg/kg-day for 26 weeks (Thomford 2001b; Butenhoff et al., 2002). At study initiation the monkeys weighed 3.2 to 4.5 kg. There were 4 monkeys in the 3 mg/kg-day group and 6 monkeys in each of the other groups. Dosing of animals in the 30 mg/kg-day dose group was stopped from days 11–21 because of toxicity. When dosing was resumed on day 22, animals received 20 mg/kg-day and this group was designated the 30/20 mg/kg-day group. At the end of the 26-week treatment period, 2 animals in the control and 10 mg/kg-day groups were observed for a 13-week recovery period.

Animals were observed twice daily for mortality and moribundity and were examined at least once daily for signs of poor health or abnormal behavior. Ophthalmic examinations were done before initiation of treatment and during weeks 26 and 40. Body weights were recorded weekly and food consumption was assessed qualitatively. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis before the start of treatment and on days 11, 31, 63, 91, 182, 217, 245 and 275. Blood samples were also taken for hormone determinations; samples were analyzed for estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, total and free thyroxin, testosterone, and cholecystokinin (CCK). Blood, urine and feces were collected during week 2 and every 2 weeks thereafter during treatment and recovery for PFOA concentration analyses.

At scheduled necropsy, liver samples were taken for determination of PFOA levels. The right lateral lobe of the liver was collected from each animal for palmitoyl CoA oxidase activity analyses, and representative samples of liver, right and left testes, and pancreas were collected from each animal for cell proliferation evaluation using proliferation cell nuclear antigen. All available bile was collected for bile acid determination. Weights of the adrenal glands, brain, epididymis, kidney, liver, pancreas, testis, and thyroid with parathyroid were recorded. The following tissues were collected for histopathology: adrenal (2), aorta, brain, cecum, colon, duodenum, epididymis (2), esophagus, eyes [preserved in Davidson's fixative (2)], femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, jejunum, kidneys (2), lesions, liver, lung, mesenteric lymph node, mammary gland, pancreas, pituitary, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis [(2) preserved in Bouin's solution], thymus, thyroid (2) with parathyroid, trachea and urinary bladder.

Two animals, one male from the 30/20 mg/kg-day dose group and one male from the 3 mg/kg-day dose group, were sacrificed in moribund condition during the study. The male in the 30/20 mg/kg-day dose group was sacrificed on day 29. This animal exhibited signs of hypoactivity, weight loss, few or no feces, low or no food consumption and the entire body was cold to the touch before death. Necropsy revealed esophageal and gastric lesions that were indicative of an injury that occurred during dosing and liver lesions that were presumed to be treatment related. The animal from the 3 mg/kg-day dose group was sacrificed on day 137. This animal showed clinical signs of limited use and paralysis of the hind limbs, ataxia and hypoactive behavior, few feces and no food consumption. The cause of death was not determined, but APFO treatment could not be ruled out.

Males given 30 mg/kg-day from days 1-11 had clinical signs of few feces and low food consumption and they lost weight during week 1 of treatment. Based on these signs, treatment was stopped on day 11 and was not resumed until day 22. When treatment was resumed, the dose was lowered to 20 mg/kg-day; this group was then designated the 30/20 mg/kg-day group. Of the remaining animals in this group, only 2 tolerated this dose level for the remaining 23 weeks of treatment. Treatment of three males given 30/20 mg/kg-day was halted on days 43 (week 7), 66 (week 10), and 81 (week 12) respectively. Clinical signs in these animals included thin appearance, few or no feces, low or no food consumption, and weight loss. The animals appeared to recover from compound-related effects within 3 weeks after cessation of treatment.

Mean body weight changes were notably lower during weeks 1 and 2 for males receiving 30 mg/kg-day. During week 2, this change was statistically significant. Treatment was stopped on day 11 and when it was resumed at 20 mg/kg-day on day 21, mean body weight changes were significantly lower than controls during weeks 7, 9 and 24. Overall mean body weight changes through week 27 were notably lower for the males in the 30/20 mg/kg-day group. There was an increased incidence of low or no food consumption for animals in the 30/20 mg/kg-day group that was considered to be treatment related.

There were no consistent or clearly dose-related effects on estron, estradiol, estriol, testosterone, or CCK levels in treated groups over time. Similarly, thyroid stimulating hormone and free and total thyroxine levels remained relatively constant and were not significantly altered throughout the study. Mean individual values for free and total triiodothyronine levels were statistically significantly decreased during the dosing period at the 30/20 mg/kg/day dose group compared to controls, although the biological significance of this decrease is unclear.

At terminal sacrifice at 26 weeks, there were statistically significant increases in mean absolute liver weights and mean liver-to-body weight percentages in all dose groups. In addition, there was a positve dose-response trend towards an increased relative liver-to-body weight, with statistical significance at the high dose group of 30/20 mg/kg/day. The increased liver weights were considered to be treatment-related. The increased liver weight was thought to be due, in part, to hepatocellular hypertrophy (as demonstrated by decreased hepatic DNA content) which in turn may be due to mitochodrial proliferation (as demonstrated by increased succinate dehydrogenase activity).

Since administration of APFO to rats results in liver, Leydig cell and pancreatic acinar cell tumors, Butenhoff et al (2002) specifically looked for markers of tumor formation in the monkeys. In the liver, there was only a two-fold increase in hepatic palmitoyl CoA oxidase activity in the 30/20 mg/kg-day group, which is consistent with reports for other species that are not particularly responsive to PPARα-agonists. Replicative DNA synthesis in the liver, an indication of cell proliferation, was not altered in the treated animals. Similarly, it has been proposed that changes associated with the pancreatic acinar cell tumors in rats include increased serum CCK concentrations and indications of cholestasis, including alkaline phosphatase, bilirubin, and bile acids; none of these changes were noted in the cynomolgus monkeys. Finally, in the rat, it has been proposed that the Leydig cell tumors are due to a sustained increase in estradiol resulting from aromatase induction. In the treated cynomolgus monkeys there were no significant changes in estradiol, estriol, or testosterone. In addition, there was no change in replicative DNA synthesis in the pancreas or testes.

At the recovery sacrifice, there were no treatment-related effects on terminal body weights or on absolute or relative organ weights indicating that the liver weight changes seen at terminal

sacrifice were reversible over time. There were no treatment-related macroscopic or microscopic changes at the recovery sacrifice.

Serum and liver concentrations of PFOA did not increase in a dose-related manner. This may have been due to saturation or attaining steady state levels in the first several weeks of the study. In addition, there was a great deal of variability in the PFOA levels. This may have been due to the method of dosing (by capsule), the timing of dosing relative to blood sample collection and gall bladder emptying, or the analytical method (± 30% for interassay, intra-assay and system). Since steady state appeared to have been reached by 4-6 weeks of dosing, the study authors calculated the mean serum levels for the period following 6 weeks; steady state serum levels were 77 ± 39, 86 ± 33 and 158 ± 100 μg/ml for the 3, 10 and 30/20 mg/kg-day groups, respectively. In the control animals, 2/3 of the serum samples contained PFOA and averaged a level of 0.203 ± 0.154 μg/ml. There was not a statistically significant difference in the mean serum levels of the 3 and 10 mg/kg-day groups, but the mean serum level in the 30/20 mg/kg-day group was significantly higher than the 3 and 10 mg/kg-day groups. At terminal sacrifice, the levels of PFOA in the liver were similar in the 3 and 10 mg/kg-day groups, and ranged from 6.29 - 21.9 μg/g. The two monkeys in the 30/20 mg/kg-day group had liver concentrations of 16 and 83.3 μg/g. After the recovery period, the serum and liver PFOA levels in the 10 mg/kg-day group had returned to baseline.

Under the conditions of the study, the LOAEL was 3 mg/kg-day (increased liver weight and possibly mortality) and a NOAEL was not established.

3.5.2 Subchronic Studies in Rodents

Christopher and Marisa (1977) administered ChR-CD mice (5/sex/group) dietary concentrations of 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm of APFO for 28 days. The animals were observed daily and body weights and food consumption were recorded weekly. At necropsy, the organs were weighed, examined for gross pathology and preserved for histopathology. All animals in the 1000 ppm and higher groups died before the end of day 9. All animals in the 300 ppm group died within 26 days except one male. One animal in each of the 30 and 100 ppm groups died prematurely. Clinical signs were observed in mice exposed to 100 ppm and higher doses of APFO. At 100 ppm some animals exhibited cyanosis on days 10 and 11 of testing, but appeared normal throughout the rest of the study. Animals fed 300 ppm exhibited roughed fur and muscular weakness as well as signs of cyanosis after 9 days of treatment. Animals fed 1000 ppm exhibited similar effects after 6 days and those receiving 3000 ppm or greater doses exhibited effects after 4 days. There was a dose-related reduction in mean body weight in all treated groups. Relative and absolute liver weights were increased in mice fed 30 ppm or more APFO. Treatment-related changes were observed in the livers among all APFO treated animals including enlargement and/or discoloration of 1 or more liver lobes. Histopathologic examination of all surviving treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver (panlobular hypertrophy) accompanied by focal to multifocal cytoplasmic lipid vacuoles of variable size which were random in distribution.

Metrick and Marias (1977) administered ChR-CD rats (5/sex/group) dietary concentrations of 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm APFO for 28 days. The animals were observed daily and body weights and food consumption were recorded weekly. At necropsy, the organs were weighed, examined for gross pathology and preserved for histopathology. All animals in the 10,000 and 30,000 ppm groups died before the end of the first week. Gross pathologic examination revealed white foci in the cortex and medulla in the kidneys of a 10,000 ppm

female. Pelvic dilation was evident in the kidneys of a control male and a 30,000 ppm female. There were no premature deaths or unusual behavioral reactions in the other groups. Food consumption was reduced in the 1000 and 3000 ppm groups. Body weight gain was reduced as dose increased. The reduction in body weight gain was statistically significant for males at 1000 ppm and males and females at 3000 ppm. Absolute liver weights were increased in males fed 30 ppm or more and females fed 300 ppm or more. Treatment-related morphologic changes were observed in the livers of all test animals. Focal to multifocal cytoplasmic enlargement of hepatocytes in the centrilobular to midzonal areas was noted in animals fed 30 to 300 ppm, and multifocal to diffuse enlargement of hepatocytes throughout the liver lobules (panlobular) was noted in animals fed 1000 ppm or higher. The hypertrophy of hepatocytes was accompanied by acidophilic degeneration and/or necrosis of scattered liver cells with no lobular distribution. The severity and degree of tissue involvement were more pronounced in males than in females.

Goldenthal (1978a) administered ChR-CD rats (5/sex/group) dietary levels of 0, 10, 30, 100, 300, and 1000 ppm APFO for 90 days. These dose levels are equivalent to 0.056, 1.72, 5.64, 17.9, and 63.5 mg/kg-day in males, and 0.74, 2.3, 7.7, 22.36 and 76.47 mg/kg-day in females. Animals were observed twice daily and body weight and food consumption were recorded weekly. Blood and urine samples were collected during the pretest period and at 1 and 3 months of the study for hematology and clinical chemistry and urinalysis. At necropsy, the organs from the control, 100, 300, and 1000 ppm groups were weighed and examined for histopathologic lesions; livers from the 10 and 30 ppm groups were also examined microscopically.

There were no treatment-related changes in behavior or appearance. One female in the 100 and one female in the 300 ppm group died during collection of blood. These deaths were not considered to be treatment related. All other animals survived until scheduled sacrifice. There was a decrease in body weight gain for male rats at the 300 and 1000 ppm dose level. At 13 weeks, mean body weight of males in the 1000 ppm group was significantly less than that of controls. There were no treatment related effects on the hematologic, biochemical or urine parameters.

Relative kidney weights were significantly increased in males in the 100, 300, and 1000 ppm groups. However, absolute kidney weights were comparable among groups, and there were no histopathological lesions. Absolute liver weights were significantly increased in males in the 30, 300 and 1000 ppm groups and in females in the 1000 ppm group. Relative liver weights were significantly increased in males in the 300 and 1000 ppm groups and in females in the 1000 ppm group. Discoloration on the surface of the liver was observed in male rats in the 1000 ppm group. Hepatocellular hypertrophy (focal to multifocal in the centrilobular to midzonal regions) was observed in 4/5, 5/5, and 5/5 males in the 100, 300, and 1000 ppm groups, respectively. Hepatocyte necrosis was observed in 2/5, 2/5, 1/5, and 2/5 males in the 30, 100, 300, and 1000 ppm groups, respectively. Under the conditions of this study, the LOAEL for males is 30 ppm (1.72 mg/kg-day) based on liver effects and the NOAEL is 10 ppm (0.56 mg/kg-day); the LOAEL for females is 1000 ppm (76.5 mg/kg-day) and the NOAEL is 300 ppm (22.4 mg/kg-day).

Palazzolo (1993) administered male ChR-CD rats (45-55 per group) dietary concentrations of 1, 10, 30, or 100 ppm APFO for 13 weeks. These doses are equivalent to 0.06, 0.64, 1.94, and 6.50 mg/kg-day. Two control groups (a nonpair-fed control group and a control group pair-fed to the 100 ppm dose group) were fed basal diet during that period. Following the 13-week exposure period, 10 animals per group were fed basal diet for an 8-week recovery period. The animals were observed twice daily for clinical signs of toxicity, and body weights and food consumption

were recorded weekly. Food consumption was recorded daily for the pair-fed animals. A total of 15 animals per group were sacrificed following 4, 7, or 13 weeks of treatment; 10 animals per group were sacrificed after 13 weeks of treatment and following the 8 week recovery period. Serum samples collected from 10 animals per group at each scheduled sacrifice during treatment and from 5 animals per group during recovery were analyzed for estradiol, total testosterone, luteinizing hormone, and PFOA. The level of palmitoyl CoA oxidase was analyzed from a section of liver that was obtained from 5 animals per group at each scheduled sacrifice. Weights of the brain, liver, lungs, testis, seminal vesicle, prostate, coagulating gland, and urethra were recorded, and these tissues were examined histologically. In addition, the brain, liver, lungs, testis, seminal vesicle, and prostate were preserved in glutaraldehyde for electron microscopic examination.

In the analysis of the data, animals in groups exposed to 1, 10, 30, and 100 ppm APFO were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to 100 ppm APFO. No treatment-related clinical signs were noted. At 100 ppm, significant reductions in body weights were seen compared to the pair-fed control group during week 1 and the nonpair-fed control group during weeks 1-13. Body weight data in the other dosed-groups were comparable to controls. At 100 ppm, mean body weight gains were significantly higher than the pair-fed control group during week 1 and significantly lower than the nonpair-fed control group during weeks 1-13. At 10 and 30 ppm, mean body weight gains were significantly lower than the nonpair-fed control group at week 2. These differences in body weight and body weight gains were not observed during the recovery period. Animals fed 100 ppm consumed significantly less food during weeks 1 and 2, when compared to the nonpair-fed control group. Overall, there was no significant difference in food consumption. There were no significant differences among the groups for any of the hormones evaluated in the serum although there appeared to be some indication of elevated estradiol for the 100 ppm group at week 5.

Significant increases in absolute and relative liver weights and hepatocellular hypertrophy were observed at weeks 4, 7, or 13 in the 10, 30 and 100 ppm groups. There was no evidence of any degenerative changes or abnormalities associated with the hypertrophy. Hepatic palmitoyl CoA oxidase activity was significantly increased at weeks 4, 7, and 13 in the 30 and 100 ppm groups. At 10 ppm, hepatic palmitoyl CoA oxidase activity was significantly increased at week 4 only. During recovery, however, none of the liver effects were observed, indicating that these treatment-related liver effects were reversible.

Serum levels from the male rats in this study are shown below in Table 17.

Table 17
Summary of Serum Values (μg/ml) in Male Rats Treated with APFO for 90 Days

Week	Dose Group (mg/kg-day)			
	0.06	0.64	1.94	6.50
5	6.5 ± 1.05[a] (8)	55.4 ± 8.08 (9)	103.5 ± 14.38 (8)	159.3 ± 30.16 (10)
8	7.5 ± 1.27 (9)	45.5 ± 16.20 (10)	87.3 ± 27.48 (10)	149.1 ± 34.98 (10)
14	7.1 ± 1.15 (10)	41.2 ± 12.98 (10)	70.3 ± 16.18 (10)	137.6 ± 33.83 (10)
22	1.2 (1)	1.09 ± 1.303 (3)	1.64 ± 0.918 (3)	2.45 ± 0.849 (2)

a - Mean ± SD, (N)

Under the conditions of this study, the LOAEL is 10 ppm (0.64 mg/kg-day) based on increases in absolute and relative liver weights with hepatocellular hypertrophy. The NOAEL is 1.0 ppm (0.06 mg/kg-day).

3.5.3 Chronic Toxicity and Carcinogenicity Studies in Rats

The chronic toxicity and carcinogenic potential of PFOA has been investigated in two dietary studies in rats. In the first study (Sibinski, 1987), groups of 50 male and 50 female Sprague-Dawley (Crl:CD BR) rats were fed diets containing 0, 30 or 300 ppm APFO for two years. Groups of 15 additional rats per sex were fed 0 or 300 ppm APFO and evaluated at the one year interim sacrifice. In males. the mean test article consumption was 1.3 and 14.2 mg/kg-day for the 30 and 300 ppm groups, respectively; in females, the mean test article consumption was 1.6 and 16.1 mg/kg-day for the 30 and 300 ppm groups, respectively. All animals were observed daily throughout the two year dosing period. Body weights and feed consumption were recorded once per week for the first six months, and then once every two weeks for the remainder of the study. Clinical pathological examinations including hematology, serum chemistry and urinalysis were conducted on samples obtained from 15 rats per sex from each group at 3, 6, 12, 18 and 24 months. Macroscopic postmortem examinations were performed on all animals that died during the study and those which were terminated at the one year interim and two year necropsies. The weights of the kidneys, liver, testes, brain, heart, spleen, adrenal glands and uterus were recorded for 15 randomly selected rats/sex at the interim termination from both the control and high-dose groups, and from the control and both treated groups at the two year necropsy. Microscopic evaluation was performed on all tissues from all of the control and high-dose rats.

There was a dose-related decrease in body weight gains in the males rats and to a lesser extent, in the female rats as compared to the controls; the decreases were statistically significant in the high-dose groups of both sexes (up to 21% in male rats and 11% in female rats). The body weight changes are treatment related since feed consumption was actually increased (rather than decreased). There were no differences in mortality between the treated and untreated groups; the

survival rates at the end of 104 weeks for the male control, low-, and high-dose groups were 70%, 72% and 88%, respectively; in females, the survival rates were 50%, 48% and 58% for the control, 30 and 300 ppm groups, respectively. The only clinical sign observed was a dose-related increase in ataxia in the female rats; which was most commonly associated with moribund animals; the incidences in the control, low- and high-dose groups were 4%, 18% and 30%. Significant decreases in red blood cell counts, hemoglobin concentrations and hematocrit values were observed in the high-dose male and female rats as compared to control values. Clinical chemistry changes included slight (less than 2-fold), but significant increases ($P< 0.05$) in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) in both treated male groups from 3-18 months, but only in the high-dose males at 24 months. Slight (up to about 10%) increases in absolute or relative liver and kidney weights were noted in both high-dose male and female rats at the 1 year interim sacrifice and at the terminal necropsy; however, only the relative liver weight (vs. body weight or brain weight) increases in the high-dose males were statistically significant ($P<0.05$).

Histologic evaluations showed lesions in the liver, testis and ovary. In the liver, the increased incidence of lesions reached statistical significance only in the high-dose male group. At the 1 year interim sacrifice, diffuse hepatomegalocytosis (12/15 animals), portal mononuclear cell infiltration (13/15 animals) and hepatocellular necrosis (6/15 animals) were seen in the high-dose males while incidences in the control group were, 0/15, 7/15 and 0/15, respectively. Hepatocellular vacuolation was seen in 11/15 high-dose females as compared to an incidence of 5/15 in the control group. At the 2-year sacrifice, megalocytosis was found at an incidence of 0%, 12% and 80% in the males, and 0%, 2% and 16% in the females from the control, low-, and high-dose groups, respectively. Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, was observed in 14% and 56% of the low- and high-dose males, as compared to a control incidence of 8%. The incidence of hyperplastic nodules, a localized proliferation of hepatic parenchymal cells, was slightly increased in the high-dosed males with an incidence of 6% as compared to 0% in the control males.

At the one-year sacrifice, testicular masses were found in 6/50 high-dose and 1/50 low-dose rats, but not in any of the controls. Furthermore, marked aspermatogenesis was found in 2/15 high-dose males but none in the control males. At the 2-year sacrifice, vascular mineralization of the testes occurred in 18% of the high-dosed male and 6% of the low-dosed males, but was not seen in the controls. These testicular effects reached statistical significance in the high-dose group.

A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats at the 2-year sacrifice. The incidence of this lesion in the control, low-, and high-dose groups was 0%, 14%, and 32%, respectively. The biological significance of this effect at the time of the initial evaluation was unknown, as there was no evidence of progression to tumors. Recently, however, slides of the ovaries from that study were re-evaluated, with particular emphasis placed on the proliferative lesions of the ovary (Mann and Frame, 2004). Using more recently published nomenclature, the ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, which corresponded to the diagnoses of tubular hyperplasia or tubular adenoma by the original study pathologist. The data are summarized in Table 18. No statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined were seen in treated groups compared to controls. There was some evidence of an increase in size of stromal lesions observed at the 300 ppm group; however, adenomas occurred in greater incidences in the control group than in either of the treated groups. Results of this follow-up evaluation indicated that rats sacrificed at the one-year interim

sacrifice, as well as rats that died prior to the interim sacrifice were not considered at risk for tumor development.

Table 18
Incidence of Ovarian Stromal Hyperplasia and Adenoma in Rats

Group	Control	30 ppm	300 ppm
No. examined	45	47	46
Hyperplasia (Total)	8	16	15
Grade 1	6	7	5
Grade 2	2	3	1
Grade 3	0	5	6
Grade 4	0	1	3
Adenoma	4	0	2
Adenoma and/or Hyperplasia	12	16	17

From Mann and Frame (2004)

Based on these toxic effects, the high dose selected in this study appears to have reached the Maximum Tolerated Dose. Based on a decrease in body weight gain, increase in liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male rats is 300 ppm and the NOAEL is 30 ppm. The LOAEL for female rats is 300 ppm based on a decrease in body weight gain and hematologic effects and the NOAEL is 30 ppm.

At the termination of the study, there was a significant increase ($P<0.05$) in the incidence of testicular (Leydig) cell adenomas in the high-dose male rats. The incidence of the Leydig cell tumors (LCT) in the control, low- and high-dose males was 0/50 (0%), 2/50 (4%) and 7/50 (14%), respectively. The increase was also statistically significant when compared to the historical control incidence of 0.82% observed in 1,340 Sprague-Dawley control male rats used in 17 carcinogenicity studies (Chandra et al., 1992). The spontaneous incidence of LCT in 2-year old Sprague-Dawley rats in other studies was reported to be approximately 5% (cited in Clegg et al., 1997).

There was also a significant increase ($P<0.05$) in the incidence of mammary fibroadenomas in both groups of female rats. The incidence of the mammary fibroadenoma was 10/47 (21%), 19/47 (40%) and 21/49 (43%) in the control, 30, and 300 ppm groups, respectively. The increase was also statistically significant when compared to the historical control incidence of 19.0% observed in 1,329 Sprague-Dawley control female rats used in 17 carcinogenicity studies (Chandra et al., 1992). The investigators did not consider the mammary fibroadenomas to be treatment related on the basis of the historical control incidence (24%) from a study of 181 female rats terminally sacrificed at 18 months, which is considered an inappropriate historical reference. When the mammary fibroadenoma incidences were compared to the historical control

incidence (37%) in 947 female rats in the Haskell Laboratory, however, there does not appear to be any compound related effect (Sykes,1987).

The induction of Leydig cell tumors was confirmed in a follow-up 2-year mechanistic study of PFOA toxicity in male Sprague-Dawley rats at a dietary level of 300 ppm (Cook et al., 1994; Biegel et al., 2001). There were 156 animals in the treatment group and 80 animals in the control group. Cage-side examinations were conducted at least once daily throughout the study. Rats were weighed once a week during the first 3 months and once every other week for the remainder of the study. Rats were euthanized at interim time points of 1, 3, 6, 9, 12, 15, 18, and 21 months. At each time point, the liver and testis from 6 rats/group were weighed and evaluated for cell proliferation. Another 6 rats/group were selected for peroxisome proliferation, and 10 rats/group for serum hormone (estradiol, testosterone, LH, FSH, and prolactin) analysis. All rats surviving the 24-month test period were necropsied for microscopic examination of various organs: e.g., kidneys, liver, testes, brain, heart, spleen.

In the treated group, relative liver weights and hepatic β-oxidation activity were statistically significantly increased at all of the sampling time points when compared to the controls. Absolute testis weights were increased only at 24 months. No hepatic or Leydig cell proliferation was observed at any of sampling times. There were no significant differences in serum testosterone, FSH, LH, or prolactin in the PFOA-treated rats when compared to the controls. There were, however, significant increases in serum estradiol concentrations in the treated rats at 1, 3, 6, 9, and 12 months.

There was a significant increase in the incidence of LCT in the treated rats (8/76, 11%) as compared to the controls (0/80, 0%). In addition, the treated group had a significant increase in the incidences of liver adenomas and pancreatic acinar cell tumors (PACT). The incidences of liver adenomas in the control and treated groups were 2/80 (3%) and 10/76 (13%), respectively, whereas those for the pancreatic acinar cell adenomas were 0/80 (0%) and 7/76 (9%). There was one pancreatic acinar cell carcinoma in 76 of the treated rats and none in 80 controls. The incidence of combined pancreatic acinar cell adenoma/carcinoma in the treated rats (8/76, 11%) was significantly increased as compared with the controls (0/80, 0%).

In the first carcinogenicity study (Sibinski, 1987), there was no reported increase in the incidence of PACT, and the incidence of pancreatic acinar hyperplasia in the male rats was 0/33, 2/34, and 1/43 in the control, 30 and 300 ppm groups, respectively. To resolve this discrepancy, the histological slides from both studies were reviewed by independent pathologists. This review of the microscopic lesions of the pancreas in the two studies indicate that PFOA produced increased incidences of proliferative acinar cell lesions of the pancreas in the rats of both studies at the dietary concentration of 300 ppm. The differences observed were quantitative rather than qualitiative; more and larger focal proliferative acinar cell lesions and greater tendency for progression of lesions to adenoma of the pancreas were observed in the second study compared to the first study. The difference between pancreatic acinar hyperplasia (reported in Sibinski, 1987) and adenoma (reported in Cook et al., 1994; Biegel et al. 2001) in the rat is a reflection of arbitrary diagnostic criteria and nomenclature by different pathologists. The basis for the quantitative difference in the lesions observed in not known but was believed to be due most likely to difference in the diets used in the two laboratories (Frame and McConnell, 2003).

In summary, the two carcinogenicity studies of PFOA have shown that PFOA induced liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumors in male Sprague-Dawley

rats. The evidence for mammary fibroadenomas in the female rats is equivocal since the incidences were comparable to some historical background incidences. PFOA has also been shown to promote liver carcinogenesis in rodents (Abdellatif et al.,1991; Nilsson et al., 1991).

3.6 Immunotoxicity Studies in Mice

Four immunotoxicity studies of PFOA have been conducted in mice. In the first study, Yang et al. (2000) administered 0.02 % PFOA to male C57Bl/6 mice in the diet for 2, 5, 7, or 10 days. At the end of the feeding period, mice were sacrificed and the liver, spleen, and thymus were weighed. The effect of PFOA administration on the cellularity, cell surface phenotype, and cell cycle of thymocytes and splenocytes was determined. In addition, effects of exposure of thymocytes and splenocytes to PFOA in vitro were examined. Administration of 0.02% PFOA for 2, 5, 7, or 10 days resulted in a significant increase, relative to controls, in liver weight, even at the earliest time point. Also, a decrease in body weight was observed. Following five days of administration, significant decreases in thymus and spleen weight were noted. After administration of 0.02% PFOA for 7 days, significant decreases (85% and 80%, respectively) in the total number of thymocytes and splenocytes were observed. In addition, the number of thymocytes expressing both CD4 and CD8 decreased by 95%; the number expressing both CD4 and CD8 decreased by 57%; and the number expressing either CD4 or CD8 decreased by 64% and 72%, respectively. For the splenocytes, both T cells (CD3) and B cells (CD19) decreased by 75% and 86%, respectively. Also, significant decreases in both CD4 helper and CD8 cytotoxic splenic T cells were observed. Upon administration of 0.02% PFOA to mice for 7 days, thymocyte proliferation was also inhibited, as detected by cell cycle flow cytometry analyses. In vitro studies showed that there was spontaneous apoptosis occurring in splenocytes and thymocytes after 8 or 24 hours of culturing in the presence of varying concentrations (50, 100, or 200 M) of PFOA. However, PFOA did not significantly alter the cell cycle under these conditions.

In order to examine the dose dependency of the effects, Yang et al. (2001) administered C57Bl/6 mice diets consisting of 0.001%-0.05% PFOA (w/w) for 10 days. For examining the time-course, a diet containing 0.02% PFOA was given for 2, 5, 7 or 10 days. Effects of withdrawal of PFOA were also studied. The results showed that, at higher doses, a significant decrease, relative to controls, in body weight was observed, although no other apparent signs of toxicity such as sores, lethargy, and poor grooming were noticed. However, a significant decrease in total water intake was observed. Mice receiving dietary PFOA for 10 days experienced significant increases in liver weight and peroxisome proliferation, as measured by induction of acyl-CoA oxidase with lauroyl-CoA or palmitoyl-CoA as substrate. These increases started at the lowest dose and reached their maximal values at a dose of 0.003-0.01%. In contrast, the weight decreases of the spleen and thymus began at a higher dose (0.01%) with no maximum reached with the doses given. The time course studies showed that increased liver weights and peroxisome proliferation were evident at the earliest time point examined. In contrast, significant thymus and spleen weight decreases required PFOA administration for a period of at least 5 days, following which the spleen weight remained constant while the thymus weight continued to decrease. However, upon prolonged treatment for one month, no further decreases in thymus and spleen weights were observed. In another set of experiments, animals received 0.02% PFOA for 7 days, and then they received normal chow for a period of 10 days. These recovery experiments showed that the animals rapidly recovered the body weight the second day after withdrawal of PFOA. However, the liver weight did not return to normal even after 10 days of recovery. Thymus recovery started on day 2 and was completed by day 10. The spleen weights returned to normal by day 2 post-withdrawal. In addition, the changes in thymus and

spleen weight upon PFOA treatment and withdrawal paralleled the changes in total thymocyte and splenocyte counts. Furthermore, flow cytometry cell cycle experiments showed that the decrease in thymocyte number caused by PFOA treatment is due mainly to inhibition of thymocyte proliferation. In contrast, PFOA treatment caused no changes in the cell cycle of splenocytes.

A third feeding study (Yang et al. 2002a) was designed to examine the possible involvement of the peroxisome proliferator-activated receptor alpha (PPARα) in the immunomodulation exerted by PFOA. This study made use of transgenic PPARα null mice, which are homozygous with regards to a functional mutation in the PPARα gene. These mice do not exhibit peroxisome proliferation or hepatomegaly and hepatocarcinogenesis even after exposure to peroxisome proliferators. These mice were fed a diet consisting of 0.02% PFOA (w/w) for 7 days. At the end of the feeding period, mice were sacrificed and the liver, spleen, and thymus were removed and weighed. The effect of PFOA on peroxisome proliferation, cell cycle, and lymphoproliferation was ascertained.

The results showed that, in contrast to wild-type mice, feeding PPARα null mice PFOA resulted in no significant decrease in body weight. As expected, peroxisome proliferation, as measured by fatty acid oxidation, was totally lacking in PPARα null mice. Also in contrast to wild type mice, feeding PPARα null mice PFOA resulted in no significant decrease in the weight of the spleen or the number of splenocytes. At the same time, the decrease in weight and cellularity of the thymus was attenuated, but not totally eliminated in the PPARα null mice. In addition, the decreases in the size of the CD4+CD8+ population of thymus cells and the number of thymus cells in the S and G2/M phases of the cell cycle, which reflects inhibition of proliferation, observed in wild type mice administered PFOA were much less extensive in PPARα null mice. Finally, in contrast to wild type mice, PFOA treatment caused no significant change in splenocyte proliferation in response to mitogens in PPARα null mice.

A fourth feeding study (Yang et al. 2002b) was designed to examine the effects of PFOA on specific humoral immune responses in mice. For this study, 0.02 % PFOA was administered to male C57Bl/6 mice for 10 days. Then the animals were examined, via plaque forming cell (PFC) and serum antibody assays, for their ability to generate an immune response to horse red blood cells (HRBCs). Ex vivo and in vitro splenic lymphocyte proliferation assays were also performed. The results showed that mice fed normal chow responded to challenge with HRBCs with a strong humoral response, as measured by the PFC assay. In contrast, mice fed with PFOA responded to HRBC immunization with no increase in HRBC-specific PFCs, relative to unimmunized controls. However, in experiments where PFOA-treated mice received normal chow following HRBC immunization, there was a significant recovery of the numbers of specific PFCs stimulated. The suppression of the humoral immune response by PFOA was confirmed by analysis of the serum anti-HRBC response. In ex vivo experiments, splenocytes isolated from control mice responded to both ConA and LPS with lymphocyte proliferation, as measured by thymidine incorporation. However, treating mice with PFOA (0.02% for 7 days) attenuated the proliferation. In a set of in vitro experiments, PFOA (1- 200 M) added to the culture medium of splenocytes cultured from untreated mice did not cause an alteration of lymphocyte proliferation in response to LPS or ConA.

3.7 Prenatal Developmental Toxicity Studies in Animals

Several prenatal developmental toxicity studies of APFO have been conducted. These include two oral studies in rats, one oral study in rabbits, and one inhalation study in rats.

Gortner (1981) administered time-mated Sprague-Dawley rats (22 per group) doses of 0, 0.05, 1.5, 5, and 150 mg/kg-day APFO in distilled water by gavage on gestation days (GD) 6-15. Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning. The remaining fetuses were subjected to skeletal examination using alizarin red.

Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15 at the high-dose group of 150 mg/kg-day. Mean maternal body weight on GD 20 continued to remain lower than controls, although the difference was not statistically significant. Other signs of maternal toxicity that occurred only at the high-dose group included ataxia and death in three rat dams. No other effects were reported. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract of the dams. Under the conditions of the study, a NOAEL of 5 mg/kg-day and a LOAEL of 150 mg/kg-day for maternal toxicity were indicated.

A significantly higher incidence in fetuses with one missing sternebrae was observed at the high-dose group of 150 mg/kg-day; however this skeletal variation also occurred in the controls and the other three dose groups (at similar incidence but lower than the high-dose group) and therefore was not considered to be treatment-related. No significant differences between treated and control groups were noted for other developmental parameters that included the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights. Likewise, a fetal lens finding initially described as a variety of abnormal morphological changes localized to the area of the embryonal nucleus, was later determined to be an artifact of the free-hand sectioning technique and therefore not considered to be treatment-related. Under the conditions of the study, a NOAEL for developmental toxicity of 150 mg/kg-day (highest dose group) was indicated.

A second oral prenatal developmental toxicity study was conducted in rabbits (Gortner, 1982). Based on the results of a range-finding study, an upper dose level of 50 mg/kg-day was set for the definitive study in which four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg-day APFO in distilled water by gavage on gestation days 6-18. Pregnancy was established in each sexually mature female by i.v. injection of pituitary lutenizing hormone in order to induce ovulation, followed by artificial insemination with 0.5 ml of pooled semen collected from male rabbits; the day of insemination was designated as day 0 of gestation. A constant dose volume of 1 ml/kg was administered. Individual body weights were measured on GD 3, 6, 9, 12, 15, 18, and 29. The does were observed daily on GD 3-29 for abnormal clinical signs. On GD 29, the does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in a 37^{0}C incubator for a 24-hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities.

Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels

on GD 12-29. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contents of the does. No clinical or other treatment-related signs were reported. Under the conditions of the study, a NOAEL of 50 mg/kg-day, the highest dose tested, for maternal toxicity was indicated.

No significant differences were noted between controls and treated groups for the number of male and female fetuses, dead or live fetuses, or fetal weights. Likewise, there were no significant differences reported for the number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate, or the 24-hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at the high-dose group (38% at 50 mg/kg-day, 30% at 5 mg/kg-day, 20% at 1.5 mg/kg-day, and 16 % at 0 mg/kg-day). A statistically significant increase in 13th ribs-spurred occurred in the mid-dose group of 5 mg/kg-day; however, the biological significance of this effect is uncertain since in both the high- and low-dose groups, this effect occurred at the same rate and was not statistically significantly different from controls. Therefore, under the conditions of the study, a LOAEL for developmental toxicity of 50 mg/kg-day (highest dose group) was indicated.

Staples et al. (1984) also conducted a developmental toxicity study of APFO in rats. The study design consisted of an inhalation and an oral portion, each with two trials or experiments. In the first trial the dams were sacrificed on GD 21; while in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35-post partum. For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to 0, 0.1, 1, 10, and 25 mg/m^3 APFO by whole-body dust inhalation for 6 hours/day, on GD 6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg-day APFO in corn oil by gavage on GD 6-15. For both routes of administration, females were mated on an as-needed basis and when the number of mated females was bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. Finally, two additional groups (six dams per group) were added to each trial that was pair-fed to the 10 and 25 mg/m^3 groups.

For trial one, the dams were weighed on GD 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Pups (live and dead) were counted, weighed and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dosed group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes.

For trial two, in which the dams were allowed to litter, the procedure was the same as that for trial one up to GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated Day 1 PP. Dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. On Day 23 PP all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. The eyes of the pups were also examined on Days 15 and 17 PP for the inhalation portion and on Days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on Day 35 PP and examined for visceral and skeletal alterations.

In trial one of the inhalation study, treatment-related clinical signs of maternal toxicity occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m^3 (on GD 12, 13, and 17). Food consumption was significantly reduced at both 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight were also observed at these concentrations, with statistical significance at the high-concentration only. Likewise, statistically significant increases in mean liver weights ($p < 0.05$) were seen at the high-concentration group. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25 mg/m^3 groups ($p = 0.002$) and in the control group pair-fed 25 mg/m^3 ($p = 0.001$). However, interpretation of the decreased fetal body weight is difficult given the high incidence of mortality in the dams. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

In trial two of the inhalation study, clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence to those described for trial one. Maternal body weight gain during treatment at 25 mg/m^3 was less than controls, although the difference was not statistically significant. In addition, 2 out of 12 dams died during treatment at 25 mg/m^3. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on Day 1 PP (6.1 g at 25 mg/m^3 vs. 6.8 g in controls). On Days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant (Day 4 PP: 9.7 g at 25 mg/m^3 vs.10.3 in controls; Day 22 PP: 49.0 g at 25 mg/m^3 vs. 50.1 in controls). No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Again, interpretation of these effects is problematic given the high incidence of maternal mortality. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

In trial one of the oral study, three out of 25 dams died during treatment of 100 mg/kg APFO during gestation (one death on GD 11; two on GD 12). Clinical signs of maternal toxicity in the dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Likewise, no significant differences between treated and control groups were noted for fetal weights, or in the incidences of malformations and variations; nor were there any effects noted following microscopic examination of the eyes.

In trial two of the oral study, similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted.

3.8 Reproductive Toxicity Studies in Animals

An oral two-generation reproductive toxicity study of APFO in rats was conducted (York, 2002; Butenhoff et al., 2004). Five groups of 30 Sprague-Dawley rats per sex per dose group were administered APFO by gavage at doses of 0, 1, 3, 10, and 30 mg/kg-day six weeks prior to and during mating. Treatment of the F0 male rats continued until mating was confirmed, and treatment of the F0 female rats continued throughout gestation, parturition, and lactation.

The F0 animals were examined twice daily for clinical signs, abortions, premature deliveries, and deaths. Body weights of F0 male rats were recorded weekly during the dosage period and then on the day of sacrifice. Body weights of F0 female rats were recorded weekly during the pre- and cohabitation periods and then on gestation days (GD) 0, 7, 10, 14, 18, 21, and 25 (if necessary) and on lactation days (LD) 1, 5, 8, 11, 15, and 22 (terminal body weight). Food consumption values in F0 male rats were recorded weekly during the treatment period, while in F0 female rats, values were recorded weekly during the precohabitation period, on GDs 0, 7, 10, 14, 18, 21, and 25 and on LDs 1, 5, 8, 11, and 15.

Estrous cycling was evaluated daily by examination of vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until confirmation of mating by the presence of sperm in a vaginal smear or confirmation of a copulatory plug. On the day of scheduled sacrifice, the stage of the estrous cycle was assessed.

For mating, one male rat and one female rat per group were cohabitated for a maximum of 14 days. Female rats with evidence of sperm in a vaginal smear or copulatory plug were designated as GD 0. Parental females were evaluated for length of gestation, fertility index, gestation index, number and sex of offspring per litter, number of implantation sites, general condition of the dam and litter during the postpartum period, litter size and viability, viability index, lactation index, percent survival, and sex ratio. Maternal behavior of the dams was recorded on LDs 1, 5, 8, 15, and 22.

F0 generation animals were sacrificed by carbon dioxide asphyxiation (day 106 to 110 of the study for male rats, i.e., after completion of the cohabitation period; and LD 22 for female rats), necropsied, and examined for gross lesions. Gross necropsy included examination of external surfaces and orifices, as well as internal examination of tissues and organs. Individual organs were weighed and organ-to-body weight and organ-to-brain weight ratios were calculated for the brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary, uterus with oviducts and cervix, left epididymis (whole and cauda), right epididymis, prostate and seminal vesicles, (with coagulating glands and with and without fluid). Tissues retained in neutral buffered 10% formalin for possible histological evaluation included the pituitary, adrenal glands, vagina, uterus, with oviducts, cervix and ovaries, right testis, seminal vesicles, right epididymis, and prostate. Histological examination was performed on tissues from 10 randomly selected rats per sex from the control and high dosage groups. All gross lesions were examined histologically. All F0 generation rats that died or appeared moribund were also examined.

Histological examination of the reproductive organs in the low- and mid-dose groups was conducted in rats that exhibited reduced fertility by either failing to mate, conceive, sire, or deliver healthy offspring; or for which estrous cyclicity or sperm number, motility, or morphology were altered. Sperm number, motility, and morphology were evaluated in the left cauda epididymis of F0 generation male rats; testicular spermatid concentrations were evaluated in the left testis. The number and distribution of implantation sites were recorded in F0

generation female rats. Rats that did not deliver a litter were sacrificed on GD 25 and examined for pregnancy status. Uteri of apparently nonpregnant rats were examined to confirm the absence of implantation sites. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Female rats without a confirmed mating date that did not deliver a litter were sacrificed on an estimated day 25 of gestation.

At scheduled sacrifice, after completion of the cohabitation period in F0 male rats and on LD 22 in F0 female rats, blood samples (10 males and 10 females each for the 10 and 30 mg/kg-day dose groups; 3 males and 3 females for the control group) were collected for analysis of PFOA.

The F1 generation pups in each litter were counted once daily. The litter sizes were not standardized on day 4 or at anytime during lactation. Physical signs (including variations from expected lactation behavior and gross external physical anomalies) were recorded for the pups each day. Pup body weights were recorded on LDs 1, 5, 8, 15 and 22. On LD 12, all F1 generation male pups were examined for the presence of nipples. Pups that died before examination of the litter for pup viability on LD 1 were evaluated for vital status at birth. Pups found dead on LDs 2 to 22 were examined for gross lesions and for the cause of death. All F1 generation rats were weaned on LD 22 based on observed growth and viability of these pups.

At weaning (LD 22), two F1 generation pups per sex per litter per group (60 male and 60 female pups per group) were selected for continued evaluation, resulting in 600 total rats (300 rats per sex) assigned to the five dosage groups. At least two male pups and two female pups per litter, when possible, were selected. F1 generation pups not selected for continued observation for sexual maturation were sacrificed. Three pups per sex per litter were examined for gross lesions. Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. The brain, spleen and thymus from one of the three selected pups per sex per litter were weighed and the brain, spleen, and thymus from the three selected pups per sex per litter were retained for possible histological evaluation. All remaining pups were discarded without further examination.

The F1 generation rats were given the same dosage level of the test substance and in the same manner as their respective F0 generation sires and dams. Dosages were given once daily, beginning at weaning and continuing until the day before sacrifice. F1 generation female rats were examined for age of vaginal patency, beginning on day 28 postpartum (LD 28). F1 generation male rats were evaluated for age of preputial separation, beginning on day 39 postpartum (LD 39). Body weights were recorded when rats reached sexual maturation.

Following sexual maturation, a table of random units was used to select one male and one female per litter per group for continuation through mating to produce the F2 generation. The remaining F1 animals were sacrificed.

Estrous cycling was evaluated daily by examination of vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until confirmation of mating by the presence of sperm in a vaginal smear or confirmation of a copulatory plug. On the day of scheduled sacrifice, the stage of the estrous cycle was assessed.

A table of random units was used to assign F1 generation rats to cohabitation, one male rat per female rat. If random assignment to cohabitation resulted in the pairing of F1 generation siblings, an alternate assignment was made. The cohabitation period consisted of a maximum of 14 days.

Body weights of the F1 generation male rats were recorded weekly during the postweaning period and on the day of sacrifice. Body weights of the F1 generation female rats were recorded weekly during the postweaning period to cohabitation, and on GDs 0, 7, 10, 14, 18, 21 and 25 (if necessary) and on LDs 1, 5, 8, 11, 15 and 22. Food consumption values for the F1 generation male rats were recorded weekly during the dosage period. Food consumption values for the F1 generation female rats were recorded weekly during the postweaning period to cohabitation, on GDs 0, 7, 10, 14, 18, 21 and 25 and on LDs 1, 5, 8, 11 and 15. Because pups begin to consume maternal food on or about LD 15, food consumption values were not tabulated after LD 15.

At scheduled sacrifice, the F1 animals were subjected to gross necropsy, and selected organs were weighed and examined histologically as described above for the F0 animals. Sperm analyses were also conducted as described for the F0 animals.

F2 generation litters were examined after delivery to identify the number and sex of pups, stillbirths, live births and gross alterations. Each litter was evaluated for viability at least twice each day of the 22-day postpartum period. Dead pups observed at these times were removed from the nesting box. Anogenital distance was measured for all live F2 generation pups on LDs 1 and 22, and F2 male pups were examined for the presence of nipples on LD 12.

3.8.1 F0 Generation

3.8.1.1 F0 Males

One F0 male rat in the 30 mg/kg-day dose group was sacrificed on day 45 of the study due to adverse clinical signs (emaciation, cold-to-touch, and decreased motor activity). Necroscopic examination in that animal revealed a pale and tan liver, and red testes. All other F0 generation male rats survived to scheduled sacrifice. Statistically significant increases in clinical signs were also observed in male rats in the high-dose group that included dehydration, urine-stained abdominal fur, and ungroomed coat.

Significant reductions in body weight and body weight gain were reported for most of the dosage period and continuing until termination of the study in the 3, 10, and 30 mg/kg-day dose groups. Absolute food consumption values were also significantly reduced during these periods in the 30 mg/kg-day dose group, while significant increases in relative food consumption values were observed in the 3, 10, and 30 mg/kg-day within those same periods.

No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed, including numbers of days to inseminate, numbers of rats that mated, fertility index, numbers of rats with confirmed mating dates during the first and second week of cohabitation, and numbers of pregnant rats per rats in cohabitation. At necropsy, none of the sperm parameters evaluated (sperm number, motility, or morphology) were affected by treatment at any dose level.

At necropsy, statistically significant reductions in terminal body weights were seen at 3, 10, and 30 mg/kg-day (6%, 11%, and 25% decrease from controls, respectively; $p \leq 0.05$). Absolute weights of the left and right epididymides, left cauda epididymis, seminal vesicles (with and without fluid), prostate, pituitary, left and right adrenals, spleen, and thymus were also statistically significantly reduced at 30 mg/kg-day. These absolute organ weight reductions are probably due to reductions in body weight and not a reflection of target organ toxicity since the organ-to-body weight ratios were either normal or significantly increased. The biological

significance of the weight changes for the adrenals, however, is unclear since dose-related histopathological changes were observed. The absolute weight of the seminal vesicles without fluid was significantly reduced in the 10 mg/kg-day dose group. The absolute weight of the liver was statistically significantly increased in all dose-groups. Absolute kidney weights were statistically significantly increased in the 1, 3, and 10 mg/kg-day dose groups, but significantly decreased in the 30 mg/kg-day group. Organ weight-to-terminal body weight ratios for the liver and for the left and right kidney were statistically significantly increased in all treated groups. The biological significance of the weight changes observed in the liver and kidney is unknown since no histopathology was conducted on these organs. Organ weight-to-terminal body weight ratios for the brain were statistically significantly increased for the 3, 10, and 30 mg/kg-day dose groups; organ weight-to-brain weight ratios were significantly reduced for some organs at the high dose group, and significantly increased for other organs among all treated groups.

No treatment-related effects were seen at necropsy or upon microscopic examination of the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolation of the cells of the adrenal cortex in 2/10 males in the 10 mg/kg-day group and 7/10 males in the 30 mg/kg-day dose group.

Serum analysis for the F0 generation males in the control, 10 and 30 mg/kg-day groups sampled at the end of cohabitation showed that PFOA was present in all samples tested, including controls. Control males had an average concentration of 0.0344$\pm$ 0.0148 μg/ml PFOA. Levels of PFOA were similar in the two male dose groups; treated males had 51.1$\pm$9.30 and 45.3$\pm$12.6 μg/ml, respectively for the 10 and 30 mg/kg-day dose groups.

3.8.1.2 F0 Females

No treatment-related deaths or adverse clinical signs were reported in parental females at any dose level. No treatment-related effects were reported for body weights, body weight gains, and absolute and relative food consumption values.

There were no treatment-related effects on estrous cyclicity, mating or fertility parameters. None of the natural delivery and litter observations were affected by treatment, that is, the numbers of dams delivering litters, the duration of gestation, the averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, dams with all pups dying, liveborn and stillborn pups viability index, pup sex ratios, and mean birth weights were comparable to controls among all treated groups.

Necropsy and histopathological evaluation were also unremarkable. Terminal body weights, organ weights, and organ-to-terminal body weight ratios were comparable to control values for all treated groups, except for kidney and liver weights. The absolute weights of the left and right kidney, and the ratios of these organ weights-to-terminal body weight and of the left kidney weight-to-brain weight were significantly reduced at the highest dose of 30 mg/kg-day. The biological significance of these weight changes is not known since histopathology was not conducted on the kidney. The ratio of liver weight-to-terminal body weight was significantly reduced at 3 and 10 mg/kg-day, but there were no effects observed at 30 mg/kg-day or in absolute liver weight.

Serum PFOA levels were analyzed from the control, 10 and 30 mg/kg-day groups on LD 22. The samples were collected 24 hours after dosing. In the controls, serum PFOA was below the

limits of quantitation (0.00528 μg/ml). Levels of PFOA found in female sera increased between the two dose groups; treated females had an average concentration of 0.37±0.0805 and 1.02±0.425 μg/ml, respectively for the 10 and 30 mg/kg-day dose groups.

3.8.2 F1 Generation

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage of male pups, litter size and average pup body weight per litter at birth. At 30 mg/kg-day, one pup from one dam died prior to weaning on lactation day 1 (LD1). Additionally, on lactation days 6 and 8, statistically significant increases in the numbers of pups found dead were observed at 3 and 30 mg/kg-day. According to the study authors, this was not considered to be treatment related because they did not occur in a dose-related manner and did not appear to affect any other measures of pup viability including numbers of surviving pups per litter and live litter size at weighing. An independent statistical analysis was conducted by US EPA (2002b). No significant differences were observed between dose groups and the response did not have any trend in dose.

The authors did not present the mean pup body weights for the male and female pups separately. Pup body weight on a per litter basis (sexes combined) was reduced throughout lactation in the 30 mg/kg-day group, and statistical significance ($p \leq 0.01$) was achieved on days 1 (9.5%), 5 (9.6%), and 8 (10.5%). Of the pups necropsied at weaning, no statistically significant, treatment-related differences were observed for the weights of the brain, spleen and thymus and the ratios of these organ weights to the terminal body weight and brain weight.

3.8.2.1 F1 Males

Significant increases in treatment-related deaths (5/60 animals) were reported in F1 males in the high dose group of 30 mg/kg-day between days 2-4 postweaning. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning.

Statistically significant increases in clinical signs of toxicity were also observed in F1 males during most of the entire postweaning period. These signs included an increased incidence of annular constriction of the tail at all doses, with statistical significance at the 1, 10, and 30 mg/kg-day; a significant increase at 10 and 30 mg/kg-day in the number of male rats that were emaciated; and a significant increase in the incidence of urine-stained abdominal fur, decreased motor activity, and abdominal distention at 30 mg/kg-day.

Statistically significant reductions in body weight gain were observed at 10 and 30 mg/kg-day during days 8-15, 22-29, 29-36, 43-50, and 50-57 postweaning. Body weight gains were also significantly reduced in the 30 mg/kg-day group on days 1-8, 15-22, 36-43, 57-64, and 64-70 postweaning. Body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. In the 3 mg/kg-day group, mean body weight gain was significantly reduced on days 43-50 and 57-64 postweaning, and mean body weights were significantly reduced on days 106 and 113 postweaning. In the 1 mg/kg-day group, mean body weight gain was significantly reduced on days 15-22 and 43-50 postweaning, and mean body weights were significantly reduced on days 50, 57, 64, 70, 99, 106 and 113 postweaning. For all groups, there was a significant, dose-related reduction in mean body weight gain for the entire dosing period (days 1-113). Absolute food consumption values were significantly reduced at 10 and 30 mg/kg-day during the entire

precohabitation period (days 1-70 postweaning), while relative food consumption values were significantly increased.

Statistically significant ($p \leq 0.01$) delays in sexual maturation (the average day of preputial separation) were observed in high-dose animals versus concurrent controls (52.2 days of age versus 48.5 days of age, respectively).

No apparent effects were observed on any of the mating or fertility parameters including fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), the number of days to inseminate, the number of rats that mated, and the number of rats with confirmed mating dates during the first week. No statistically significant, treatment-related effects were observed on any of the sperm parameters (motility, concentration, or morphology).

Necroscopic examination revealed statistically significant treatment-related effects at 3, 10, and 30 mg/kg-day ranging from tan areas in the lateral and median lobes of the liver to moderate to slight dilation of the pelvis of one or both kidneys.

Statistically significant, dose-related decreases in terminal body weights were observed in the 1, 3, 10, and 30 mg/kg-day dose groups(6%, 6%, 11%, and 22% decrease from controls, respectively; $p \leq 0.01$ at 1 and 3 mg/kg-day, $p \leq 0.05$ at 10 and 30 mg/kg-day). The absolute and relative weights of the liver were statistically significantly increased in all treated groups ($p \leq 0.01$) and was accompanied by histopathological changes. The absolute weights of the left and/or right kidneys were statistically significantly increased in the 1 and 3 mg/kg-day dose groups and statistically significantly decreased in the 30 mg/kg-day dose group. Organ weight-to-terminal body weight and brain weight ratios for the kidney were statistically significantly increased in all treated groups. The biological significance of the effects on kidney weight is unknown since histopathology was not conducted on that organ. All other organ weight changes observed (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes, and epididymis) are probably due to decrements in body weight and not a reflection of target organ toxicity since the absolute weights of these organs was significantly reduced while the relative weights were either normal or significantly increased. However, the biological significance of the weight changes observed in the adrenal is unclear since histopathological changes were also observed.

Histopathologic examination of the reproductive organs was unremarkable; however, treatment-related microscopic changes were observed in the adrenal glands of high-dose animals (cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex) and in the liver of animals treated with 3, 10, and 30 mg/kg-day (hepatocellular hypertrophy). No other treatment-related effects were reported.

3.8.2.2 F1 Females

A statistically significant increase in treatment-related mortality (6/60 animals) was observed in F1 females on postweaning days 2-8 at the highest dose of 30 mg/kg-day. No adverse clinical signs of treatment-related toxicity were reported for any dose level during any time of the study period.

Statistically significant decreases in body weights were observed in high-dose animals on days 8, 15, 22, 29, 50, and 57 postweaning, during precohabitation (recorded on the day cohabitation began, when F1 generation rats were 92-106 days of age), and during gestation and lactation.

Body weight gain was significantly reduced during days 1-8 and 8-15 postweaning. Statistically significant decreases in absolute food consumption were observed during days 1-8, 8-15, and 15-22 postweaning, during precohabitation and during gestation and lactation in animals treated with 30 mg/kg-day. Relative food consumption values were comparable across all treated groups.

Statistically significant ($p \leq 0.01$) delays in sexual maturation (the average day of vaginal patency) were observed in high-dose animals versus concurrent controls (36.6 days of age versus 34.9 days of age, respectively).

Prior to mating, the study authors noted a statistically significant increase in the average numbers of estrous stages per 21 days in high-dose animals (5.4 versus 4.7 in controls). For this calculation, the number of independent occurrences of estrus in the 21 days of observation was determined. This type of calculation can be used as a screen for effects on the estrous cycle, but a more detailed analysis should then be conducted to determine whether there is truly an effect. 3M Company (2002) recently completed an analysis that showed there were no effects on the estrous cycle; there were no differences in the number of females with ≥ 3 days of estrus or with ≥ 4 days of diestrus in the control and high dose groups. Analyses conducted by the US EPA (2002a) also demonstrated that there were no differences in the estrous cycle among the control and high dose groups. The cycles were evaluated as having either regular 4-5 day cycles, uneven cycling (defined as brief periods with irregular pattern) or periods of prolonged diestrus (defined as 4-6 day diestrus periods) extended estrus (defined as 3 or 4 days of cornified smears), possibly pseudopregnant, (defined as 6-greater days of leukocytes) or persistent estrus (defined as 5-or greater days of cornified smears). The two groups were not different in any of the parameters measured. Thus, the increase in the number of estrous stages per 21 days that was noted by the study authors is due to the way in which the calculation was done, and is not biologically meaningful.

No effects on any of the mating and fertility parameters (numbers of days in cohabitation, numbers of rats that mated, fertility index, rats with confirmed mating dates during the first week of cohabitation and number of rats pregnant per rats in cohabitation).

All natural delivery observations were unaffected by treatment at any dose level. Numbers of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, dams with all pups dying and liveborn and stillborn pups were comparable among treated and control groups.

The terminal body weights and absolute and relative pituitary weights are shown in Table 19. No treatment-related effects were observed in the terminal body weights of the F1 females. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio, and the pituitary weight-to-brain weight ratio were statistically significantly decreased at 3 mg/kg-day and higher. Since there is not a clear dose-response relationship and histologic examination did not reveal any lesions, the biological significance of the pituitary weight data is problematic. No other differences were reported for the absolute weights or ratios for other organs evaluated. No treatment-related effects were reported following macroscopic and histopathologic examinations.

Table 19
Summary of Body and Pituitary Weights of F1 Females

Group	Terminal Body Weight (g)[a]	Pituitary Weight (g)[a]	Pituitary/ Body Weight (%)[a]	Pituitary/Brain Weight (%)[a]
Control	322.9 ± 23.4	0.017 ± 0.004	5.46 ± 1.6	0.84 ± 0.21
1 mg/kg-day	321.7 ± 24.2	0.016 ± 0.003	5.0 ± 0.86	0.76 ± 0.14
3 mg/kg-day	329.2 ± 21.5	0.015 ± 0.003*	4.65 ± 0.86*	0.74 ± 0.14*
10 mg/kg-day	325.1 ± 23.5	0.015 ± 0.002*	4.72 ± 0.90*	0.72 ± 0.12*
30 mg/kg-day	315.7 ± 20.9	0.015 ± 0.003*	4.75 ± 1.03*	0.72 ± 0.16**

a- Mean ± SD
* Significantly different from control at $p \leq 0.05$
** Significantly different from control at $p \leq 0.01$

3.8.3 F2 Generation

No treatment-related adverse clinical signs were observed at any dose level. Dead or stillborn pups were noted in both the control and treated groups. The deaths occurred on lactation days 1-18 with the majority occurring on days 1-6. However, there was no dose-response relationship and therefore were unlikely related to treatment. Statistically significant increases ($p \leq 0.01$) in the number of pups found dead were observed on lactation day 1 in the 3 and 10 mg/kg-day groups. According to the study authors, this was not considered to be treatment related because they did not occur in a dose-related manner and did not appear to affect any other measures of pup viability including numbers of surviving pups per litter and live litter size at weighing. An independent statistical analysis was conducted by US EPA (2002b). No significant differences were observed between dose groups and the response did not have any trend in dose.

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage of male pups, litter size and average pup body weight per litter when measured on LDs 1, 5, 8, 15, or 22. Anogenital distances measured for F2 male and female pups on LDs 1 and 22 were also comparable among the five dosage groups and did not differ significantly.

Terminal body weights in F2 pups were not significantly different from controls. Absolute weights of the brain, spleen and thymus and the ratios of these organ weights-to-terminal body weight and to brain weight were also comparable among treated and control groups. There were no treatment-related effects following necroscopic examination, with the exception of no milk in the stomach of the pups that were found dead.

3.8.4 Conclusions

Dosing with APFO at 30 mg/kg-day resulted in a delay in the onset of sexual maturation in both male and female F1 offspring. The authors of the study contend that the delays in sexual maturation (preputial separation or vaginal patency) observed in high-dose animals are due to the

fact that these animals have a decreased gestational age, a variable which they have defined as the time in days from evidence of mating in the F0 generation until evidence of sexual maturation in the F1 generation. The authors state that gestational age appeared to be decreased in high-dose animals at the time of acquisition (the time when sexual maturation was reached), which they believe meant the animals in that group were younger and more immature than the control group, in which there was no significant difference in sexual maturation.

In order to test this hypothesis, the authors covaried separately the decreases in body weight and in gestational age with the delays in sexual maturation in order to determine whether or not body weights and gestational age were a contributing factor. When the body weight was covaried with the time to sexual maturation, the time to sexual maturation showed a dose related delay that was statistically significant at $p \leq 0.05$. This suggests that the delay in sexual maturation was partly related to body weight, but not entirely. When gestational age was covaried with the time to sexual maturation, there was no significant difference in the time of onset of sexual maturation between controls and high-dose animals. This indicates that the effect of delayed sexual maturation could possibly be attributed to decreased gestational age.

While it is known and commonly accepted that changes in the body weights of offspring can affect the time to sexual maturation, whether or not gestational age, as defined by the authors, also affects the time of sexual maturation is purely speculative, especially since there were no data provided by the authors to support this relationship. Additionally, covaring gestational age with time to sexual maturation is problematic from a statistical standpoint. Since there was no significant change in the length of gestation at 30 mg/kg-day, based on the authors' definition of 'gestational age', the decreases in gestational age would have to be due mostly to changes in time to sexual maturation. Therefore, sexual maturation is essentially being covaried with itself. Still, even if a relationship between gestational age and time to sexual maturation were shown, it merely offers an explanation for the observed delays in sexual maturation in high-dose animals, but does not diminish its significance.

A variety of endpoints are evaluated throughout different lifestages in a two generation reproductive toxicity study. Therefore, some of the endpoints may be indicative of developmental/reproductive toxicity, while others may be indicative of adult toxicity. The selection of developmental endpoints (and the appropriate LOAELs and NOAELs) for this study was based on the Agency's Developmental Toxicity Risk Assessment Guidelines (EPA, 1991) and the Agency's Reproductive Toxicity Risk Assessment Guidelines (EPA, 1996). According to the guidelines, the period of exposure for developmental toxicity is prior to conception in either parent, through prenatal development and continuing until sexual maturation. In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism. For selection of the developmental endpoints from the two generation reproductive toxicity study, attention was focused on effects that were noted during the period of developmental exposure. Thus, only effects that occurred up to sexual maturation were considered relevant for assessing developmental toxicity. Effects occurring after sexual maturation were considered relevant for assessing adult toxicity since it was not possible to determine whether the effects were due to developmental and/or adult exposures.

Therefore, under the conditions of the study, the LOAEL for F0 parental males is 1 mg/kg-day, the lowest dose tested, based on significant increases in absolute and relative liver weight. A NOAEL for the F0 parental males could not be determined since the increases in liver weight were seen at all doses tested. The NOAEL for F0 parental females is 30 mg/kg-day, the highest dose tested.

A variety of developmental/reproductive effects were noted in the F1 generation. During lactation, there was a significant reduction in F1 mean body weight on a litter basis (sexes combined) in the 30 mg/kg-day group. F1 males in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day beginning on postweaning day 8. F1 females in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 males and females in the 30 mg/kg-day group. For F1 males, the LOAEL for developmental/reproductive toxicity was 10 mg/kg-day, and the NOAEL was 3 mg/kg-day. For F1 females, the LOAEL for developmental/reproductive toxicity was considered to be 30 mg/kg-day, and the NOAEL was 10 mg/kg-day.

The LOAEL for adult systemic toxicity in the F1 males is 1 mg/kg-day based on significant, dose-related decreases in body weights and body weight gains (observed prior to and during cohabitation and during the entire dosing period), and in terminal body weights; and significant changes in absolute and relative liver weights. A NOAEL for the F1 males could not be determined since these effects were seen at all doses tested.

The NOAEL and LOAEL for adult systemic toxicity in the F1 females are 10 and 30 mg/kg-day, respectively, based on statistically significant decreases in body weight and body weight gains.

The NOAEL for developmental/reproductive toxicity in the F2 offspring was 30 mg/kg-day. No treatment-related effects were observed at any doses tested in the study. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation.

3.9 Mode of Action and Summary of Weight of Evidence

3.9.1 Epidemiology Studies

All of the epidemiologic data available on PFOA are based on occupational studies, most of which were routine biomonitoring efforts conducted by 3M Company. With the exception of one, all of the studies were cross-sectional and mostly analyzed males, the majority of workers at these plants. Consequently, reproductive and developmental outcomes have not been studied.

In the 3M mortality studies, the only statistically significant association reported was for prostate cancer mortality and employment duration in a plant that manufactures PFOA; however, this association was not observed in a follow-up study which included more specific exposure measures (Gilliland and Mandel, 1993; Alexander, 2001a). In the Dupont cancer study, bladder and kidney cancer incidence was elevated among employees (Dupont, 2003). However, very little other data on exposures, including other chemicals at the plant, were available. A study on hormone levels in male workers indicated an increase in estradiol levels in workers with the highest PFOA serum levels; however, the results may have been confounded by body mass index (Olsen, et al., 1998a). There were no changes of note in other hormone levels in these workers. Another study of CCK levels in employees, in which increased levels have been linked to pancreas acinar cell adenomas in rats, did not report increases in workers (Olsen, et al., 1998b; Olsen et al., 2000). In addition, cholesterol and triglyceride levels in workers were positively associated with PFOA exposures, inconsistent with the hypolipidemic results reported in rat

studies. There was also a statistically significant positive association reported for PFOA and T3 but not any other thyroid hormones (Olsen, et al., 2001e).

Overall, there were no notable health effects reported in fluorochemical workers which can be directly attributed to PFOA exposure. However, there were many limitations in these studies (detailed in Section 3.1) which need to be considered.

3.9.2 Metabolism and Pharmacokinetics

Little information is available concerning the pharmacokinetics of PFOA and its salts in humans. An ongoing 5-year, half-life study in 7 male and 2 female retired workers has suggested a mean serum PFOA half-life of 4.37 years (range, 1.50 – 13.49 years). Metabolism and pharmacokinetic studies in non-human primates are limited to a study of 3 male and 3 female cynomolgus monkey administered a single IV dose of 10 mg/kg potassium PFOA. In male monkeys, the average serum half life was 20.9 days. In female monkeys, the average serum half life was 32.6 days.

Studies in adult rats have shown that PFOA is absorbed following oral, inhalation, and dermal exposures. Serum pharmacokinetic parameters of PFOA have been evaluated in adult Sprague-Dawley rats following gavage administration, and in Wistar rats following IV administration. The distribution of PFOA has been examined in tissues of adult rats following administration by gavage and by i.v. and i.p. injection. PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. Although investigated in several studies, there is no evidence that PFOA is metabolized. In adult rats, there is evidence of enterohepatic circulation of PFOA. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats. There are gender differences in the elimination of PFOA in rats. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8 to 16 hours, while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138 to 202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

No studies have been conducted to specifically examine the absorption, metabolism or elimination of PFOA in the developing rat. However, recent studies have been conducted to examine the concentrations of PFOA in the developing Sprague-Dawley rat, and to determine when the gender difference in elimination of PFOA becomes apparent. In addition, several studies have examined the serum and tissue distribution of PFOA in newly weaned Wistar rats. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. During lactation and for the first several weeks after weaning, the elimination of PFOA is similar in males and females. Between 4-5 weeks of age, the elimination in male rats assumes the adult pattern and the gender difference becomes readily apparent. Distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

It has been suggested that PFOA is circulated around the body by noncovalently binding to plasma proteins. Several studies have investigated the binding of PFOA to plasma proteins of

rats, humans or monkeys to gain understanding of its absorption, distribution and elimination and species and gender differences. There is limited additional information on the metabolism and pharmacokinetics of PFOA in mice, rabbits and dogs.

3.9.3 Mode of Action Analyses and Cancer Descriptor

There has been a great deal of scientific debate and research into rodent liver toxicity and liver tumors that are induced through the activation of PPARα, and several groups of scientists have summarized the state of the science over the years (IARC, 1995; Cattley et al. 1998). More recently, the ILSI Risk Science Institute convened a workgroup to re-examine the mode of action of PPARα agonist-induced rodent liver tumors as well as to evaluate the mode(s) of action for Leydig cell and pancreatic acinar cell tumors, which also are observed frequently in rats with PPARα agonists (Klaunig et al., 2003). This effort utilized and extended guidance that the US EPA (1999) and IPCS (Sonich-Mullin et al., 2001) have provided for assessing information for the delineation of an animal mode of action (MOA) for specific tumor types. This guidance is also applicable for assessing MOAs for non-tumor responses. In this context, it is important to note that the term "mode of action" is defined as the sequence of key cellular and biochemical events (measurable parameters) that result in a toxicological effect. The analysis does not require knowledge of "mechanism of action" which implies a more detailed understanding of the molecular basis of the toxicological effect.

Recently, EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS) presented a draft of a proposed OPPTS science policy on PPARα mediated hepatotoxicity and heptacarcinogenesis to the FIFRA Science Advisory Panel (SAP) in December, 2003 (OPPTS, 2003). The OPPTS guidance document describes the approach the Office proposes to use to evaluate the scientific information regarding the mode of action of PPARα agonists in rodent hepatocarcinogenesis and the relevance of this mode of action for human hepatocarcinogenesis. Other tumor types (e.g. Leydig cell and pancreatic acinar cell tumors) that may be associated with PPARα-agonists are also briefly described. The document provides an overview of the evidence for a PPARα-agonist mode of action for liver tumors in rodents, and an overview of all known age and species differences in the key events. The document also provides proposed guidance on the data needed to demonstrate that rodent liver toxicity and tumors have arisen as a result of a PPARα agonist mode of action, and the relevance of this mode of action for humans.

As described in section 3.5, several studies of PFOA in adult rats have shown that the liver is a principle target organ, and long-term exposure results in liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumors. The mode of action information for each of these effects are described below and are described in the context of the work of Klaunig et al. (2003), the proposed OPPTS science policy and the response from the FIFRA SAP (FIFRA SAP, 2004).

3.9.3.1 Mode of Action Analysis of Liver Toxicity and Liver Adenomas in Rats

There are a number of possible modes of action for hepatocarcinogenesis of chemicals. As summarized in Section 3.4, the weight of evidence from short-term genotoxicity assays suggest that PFOA is not a DNA-reactive compound.

As mitochondria play a major role in cell signaling and apoptotic modes of cell death, and several structurally related perfluorinated compounds have been shown to manifest their toxicity by interfering with mitochondria biogenesis and bioenergetics, the effects of PFOA on mitochondrial biogenesis and bioenergetics were investigated. A number of studies have shown

that mitochondria biogenesis in liver was increased following treatment of rats with PFOA (*e.g.*, Berthiaume and Wallace, 2002). PFOA has also been demonstrated to uncouple oxidative phosphorylation in mitochondria of the liver from rats exposed to PFOA in the diet (Keller et al., 1992). At high concentrations, PFOA caused a small increase in resting respiration rate and slightly decreases the membrane potential. The observed effects are believed to be attributed to a slight increase in nonselective permeability of the mitochondria membranes caused by the surface-active property of the compound (Starkov and Wallace, 2002). Treatment with 200 μM PFOA was found to cause a dramatic increase in the cellular content of reactive oxygen species (ROS) in human hepG2 cells. The activation of caspase-9 and apoptosis by PFOA observed was postulated to be the result of the disruption of mitochondria membrane and accumulation of ROS (Panaretakis et al., 2001). Further research is needed, however, to elucidate how apoptosis is involved in tumorigenesis of PFOA.

Gap junctional intercellular communication (GJIC), a process by which cells exchange ions, second messages, and other small molecules, is important for normal growth, development, and differentiation, as well as maintenance of homeostasis in muticellular organisms. Because tumor formation requires loss of homeostasis and many tumor promoters inhibit GJIC, it has been hypothesized that GJIC may play a role in carcinogenesis (Trosko et al., 1998). PFOA has been demonstrated to inhibit GJIC in liver cells in vitro and in vivo (Upham et al., 1998). Since inhibition of GJIC is a widespread phenomenon, and the effect by PFOA was neither species nor tissue specific and was reversible, the significance of GJIC inhibition in regard to the mode of carcinogenic action of PFOA is unknown.

Estrogen has been shown to promote hepatocarcinogenesis in rats (Yager and Yager, 1980; Cameron et al., 1982). However, more research is needed to support the involvement of this MOA in the hepatocarcinogenesis of PFOA.

Low and sporadic incidences of liver necrosis were noted in both control and treated rats in the subchronic and chronic toxicity studies of PFOA. Liver necrosis and regenerative cell proliferation may play a role in promoting pre-initiated cells in carcinogenesis. The involvement of necrosis in the liver tumor induction by PFOA in rats remains to be investigated.

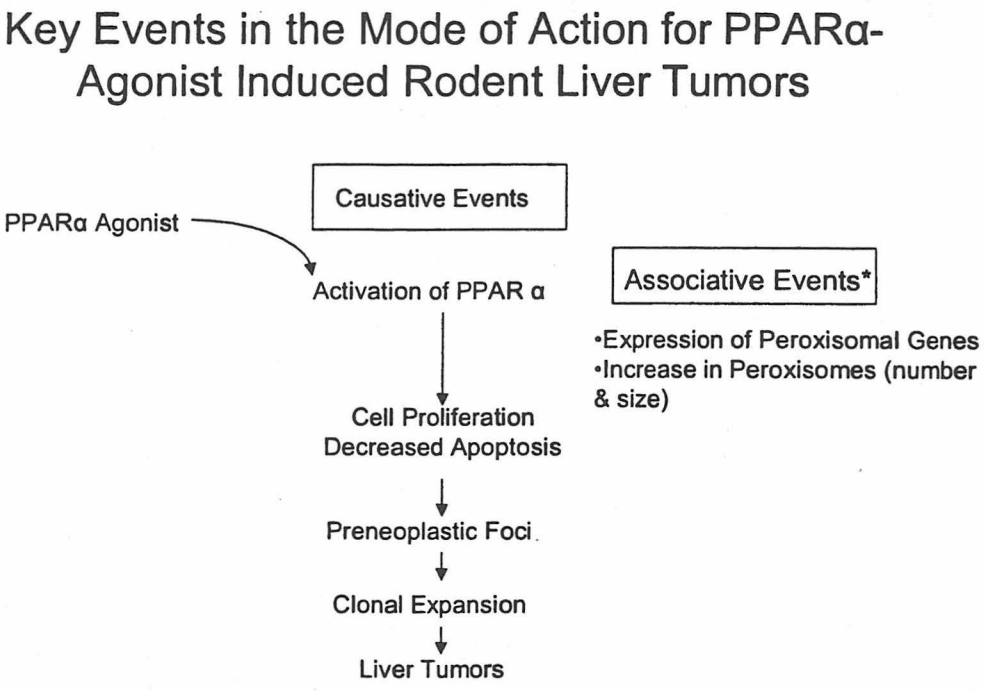
While available data are not sufficient to support any of the above MOAs for the liver tumor induction by PFOA, there is strong evidence to conclude that the liver toxicity and liver adenomas that are observed in rats following exposure to PFOA result from a PPARα-agonist MOA. As described in Klaunig et al. (2003) and OPPTS (2003), the MOA of PPARα-agonist induced liver toxicity and liver tumors involves four causal key events which are shown in Figure 1. (Page 78, below.) The first key event is activation of PPARα (which regulates the transcription of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism). Activation of PPARα leads to an increase in cell proliferation and a decrease in apoptosis, which in turn leads to preneoplastic cells and further clonal expansion and formation of liver tumors. Of these key events, only PPARα activation is highly specific for this MOA while cell proliferation/apoptosis and clonal expansion are common to other modes of action. As depicted in Figure 1, there are also several "associative" events that are markers of PPARα agonism but are not directly involved in the etiology of liver tumors. These include peroxisome proliferation (a highly specific indicator that this MOA is operative) and peroxisomal gene expression. Peroxisomal proliferation may also result in hepatocyte oxidative stress which may contribute to the mode of action by causing indirect DNA damage and leading to mutations, or by stimulating cell proliferation. However, increases in oxidative damage to DNA have not been unambiguously demonstrated for PPARα agonists. Oxidative stress is a general phenomenon,

and thus does not represent a highly specific marker for PPARα-agonist induced liver carcinogenesis.

OPPTS (2003) provided some guidance on information that would help establish that a chemical is inducing liver toxicity and tumors via a PPARα agonist MOA. This includes in vitro evidence of PPARα agonism (i.e., evidence from an in vitro receptor assay), *in vivo* evidence of an increase in number and size of peroxisomes, increases in the activity of acyl CoA oxidase, and hepatic cell proliferation. The in vivo evidence should be collected from studies designed to provide the data needed to show dose-response and temporal concordance between precursor events and liver tumor formation. Other information that is desirable and may strengthen the weight of evidence for demonstrating that a PPARα agonist MOA is operative includes data on hepatic CYP4A1 induction, palmitoyl CoA activity, hepatocyte hypertrophy, increase in liver weights, decrease in the incidence of apoptosis, increase in microsomal fatty acid oxidation, and enhanced formation of hydrogen peroxide.

There is sufficient information to demonstrate the key events for a PPARα agonist MOA following exposure to PFOA in rodents. It has been well documented that PFOA is a potent peroxisome proliferator, inducing peroxisome proliferation in the liver of rats and mice (e.g., Ikeda et al., 1985; Pastoor et al., 1987; Sohlenius et al., 1992). Maloney and Waxman (1999) demonstrated that PFOA activates PPARα using COS1 cells transfected with a luciferase reporter gene. Maximal transcriptional activity with PFOA was seen at 10 μM in the mouse PPARα (and at 20 μM in human PPARα). Like many other peroxisome proliferators, PFOA has also been shown to cause hepatomegaly (an early biomarker of peroxisome proliferator hepatocarcinogenesis) in rats (Takagi et al., 1992; Cook, 1994) and mice (Kennedy, 1986), and induce oxidative DNA damage in liver of rats (Takagi et al., 1991).

Figure 1



*Although there are other biological events (e.g., Kupffer cell mediated events, inhibition of gap junctions), the measurements of peroxisome proliferation and peroxisomal enzyme activity (in particular acyl-CoA) are widely used as reliable markers of PPARα activation.

From OPPTS (2003)

Several studies have been conducted to examine the dose-response and temporal relationships among key endpoints. The temporal and dose-response relationship of measures of peroxisome proliferation, hepatocellular hypertrophy, liver weight, and liver histopathology have been examined in male Sprague-Dawley rats following 4, 7 and 13 weeks of administration of dietary PFOA at doses ranging from 0 - 6.5 mg/kg-day (Palazzolo, 1993). The results are summarized in Table 20. There was no evidence of peroxisome proliferation, hepatocellular hypertrophy or liver weight increases at 0.06 mg/kg-day. At doses ranging from 0.64 to 6.5 mg/kg-day there is a clear relationship between peroxisome proliferation (indicated by increased palmitoyl CoA oxidase activity), hepatocellular hypertrophy and increases in liver weight at all time points. There was no evidence of hepatocellular necrosis.

Table 20
Summary of Liver Effects in Male Sprague-Dawley Rats Fed APFO for 90 Days

Parameter	Week	Dose (mg/kg-day)[1]					
		0 [a]	0 [b]	0.06	0.64	1.94	6.5
Palmitoyl CoA Oxidase (IU/G)	4 7 13	8 (0.5) 7 (1.5) 8 (0.9)	5 (0.4) 7 (1.5) 5 (1.1)	9 (1.7) 7 (0.8) 8 (1.9)	14 (3.8)[c] 18 (5.5) 10 (2.1)	24 (11.4)[c] 32 (12.2)[c] 14 (3.4)[c]	37 (14.8)[cd] 54 (35.3)[cf] 17 (4.5)[cd]
Hepatocellular Hypertrophy	4 7 13	0/15 0/15 0/15	0/15 0/15 0/15	0/15 0/15 0/15	12/15 12/15 13/15	15/15 15/15 14/15	14/15 15/15 15/15
Hepatocellular Necrosis, Coagulative	4 7 13	0/15 0/15 0/15	1/15 0/15 0/15	0/15 0/15 1/15	0/15 0/15 0/15	1/15 0/15 1/15	2/15 1/15 0/15
Absolute Liver Weight (g)	4 7 13	16.34 (2.14) 17.78 (2.12) 19.73 (2.01)	15.83 (1.13) 16.91 (2.22) 16.30 (1.62)	15.45 (1.71) 17.68 (??) 18.03 (2.81)	17.89 (2.13) 19.42 (2.10) 20.44 (2.87)	23.23 (2.83)[c] 27.76 (3.51)[c] 22.74 (4.21)	25.44 (1.89)[cd] 27.76 (3.51)[cd] 26.78 (5.47)[cd]
Liver/Body Weight (%)	4 7 13	3.97 (0.37) 3.75 (0.29) 3.53 (0.28)	4.07 (0.27) 3.76 (0.37) 3.23 (0.23)	3.73 (0.23) 3.64 (0.33) 3.24 (0.30)[e]	4.48 (0.32)[d] 4.12 (0.37) 3.69 (0.32)	5.77 (0.60)[d] 5.14 (0.53)[c] 4.21 (0.56)[c]	6.73 (0.49)[de] 6.06 (0.59)[cd] 5.49 (0.84)[cd]

1- Mean (SD)
a - non-pair-fed controls
b - pair-fed controls
c - statistically significant at $p < 0.05$ with the non-pair-fed control
d - statistically significant at $p < 0.05$ with the pair-fed control
e - calculated using the non-pair-fed control
f - calculated using the pair-fed control

Liu et al. (1996) characterized the dose-response relationships of several key endpoints in male CD rats exposed to doses of 0.2, 2, 20, and 40 mg/kg-day PFOA for 14 days. These endpoints included liver weight, hepatic β-oxidation, hepatic aromatase (P450 19A1), and hepatic total cytochrome P450. The NOEL for these endpoints was found to be 0.2 mg/kg-day with significant changes observed at ≥ 2 mg/kg-day for all endpoints. Thus, the studies of Palazzolo (1993) and Liu et al. (1996) demonstrate evidence of peroxisome proliferation at doses close to and below doses that result in liver adenomas following chronic exposures (1.5 and 15 mg/kg-day).

Biegel et al. (2001) examined the temporal relationship between relative liver weights, hepatic β-oxidation, and hepatic cell proliferation, and hepatic adenomas were evaluated in CD rats following PFOA exposure for 1, 3, 6, 9, 12, 15, 18, 21, and 24 months. Relative liver weights and hepatic β-oxidation were increased at all time points. Hepatic cell proliferation was numerically increased relative to the pair-fed control at 9, 15, 18, and 21 months. The liver endpoints (weight, β-oxidation, and cell proliferation) were all elevated well before the first occurrence of liver adenomas, which occurred after 12 months of treatment.

In summary, these data clearly demonstrate that PFOA induces liver toxicity and adenomas via a PPARα agonist MOA in rats. PFOA activates the PPARα and the requisite dose-response and/or temporal associations of the key events for the PPARα mode of action with the liver adenomas have been characterized.

3.9.3.2 Human Relevance of the Rat PPARα-agonist Induced Liver Toxicity and Liver Adenomas

There has been substantial scientific interest regarding the role of peroxisome proliferation in rodent hepatocarcinogenesis and its relevance for human carcinogenesis. Several scientific groups have examined the state of the science on PPARα agonist-induced rodent liver tumors over the years. In 1995, a workgroup convened under the auspices of the International Agency for Research on Cancer concluded that the MOA for liver tumors induced in rodents by PPARα agonists is unlikely to be operative in humans (IARC, 1995). The participants of a workshop held in 1998 under the auspices of the International Life Sciences Institute Health and Environmental Sciences Institute concluded that although it appeared unlikely that PPARα agonists could induce liver tumorigenesis in humans, the possibility could not be ruled out (Cattley et al. 1998). A recent analysis by Klaunig et al. (2003) concluded that: (1) the weight of evidence in linking PPARα to the mode of carcinogenic action of PFOA is high for the liver; (2) the PPARα MOA is plausible in humans since the PPARα (hPPARα) is present in humans and human livers possess PPARα at sufficient levels to mediate the hypolipidaemic response to therapeutic fibrate drugs, many of which are PPARα agonists; and (3) the weight of evidence, however, suggests that this MOA is unlikely to occur in humans based on quantitative differences in several of the key factors. For instance, human livers have been found to have 10-fold less mRNA for PPARα compared with the rodents (Palmer et al., 1998; Tugwood et al., 1998). A recent study using a PPARα-humanized mouse in which the human PPARα was expressed in the mouse liver has demonstrated that following activation by the potent ligand Wy-14643, the PPARα-mediated pathways controlling lipid metabolism are independent from those controlling the cell proliferation pathways, and suggested that structural differences between human and mouse PPARα may be responsible for the differential susceptibility to liver tumor development of fibrates (Cheung et al., 2004).

There are several additional pieces of evidence on PFOA specifically which also suggest that this MOA is not operative in non-human primates or humans. Maloney and Waxman (1999) demonstrated that PFOA activated the mouse PPARα to a greater extent than the human PPARα suggesting that humans would be less responsive to PFOA than rodents. In addition, there was no indication of hepatic cell proliferation in a 6-month cynomolgus monkey study (Butenhoff et al., 2002). Although limited, available human data have not shown an association between PFOA exposure and liver effects.

On the question of the relevance of this mode of action to humans, the majority of the SAP Panel (FIFRA SAP, 2004) agreed that there are relevant data indicating that humans are less sensitive than rodents to the hepatic effects of PPARα agonists, and concurred with the premise that when liver tumors are observed in long term studies in rats and mice, and 1) the data are sufficient to establish that the liver tumors are a result of a PPARα agonist MOA and 2) other potential MOAs have been evaluated and found not to be operative, the evidence of liver tumor formation in rodents should not be used to characterize potential human hepatocarcinogenic hazard.

3.9.3.3 Leydig Cell Adenomas in Rats

A series of mechanistic studies have been conducted (Cook et al., 1994; Biegel et al., 1995; Liu et al., 1996) to investigate the MOA associated with LCT in male Sprague-Dawley rats exposed to PFOA. Two MOAs have been proposed. One involves inhibition of testosterone biosynthesis, leading to an imbalance of androgen/estrogen levels. This leads to an increase in leutinizing hormone (LH) which promotes the development of LCT. The second MOA involves an increase in serum estradiol levels via induction of hepatic aromatase activity. Estradiol stimulates the production of growth factors such as the transforming growth factor α (TGF α) which induces Leydig cell proliferation.

Administration of PFOA to adult male rats by gavage for 14 days was shown to decrease testosterone levels and increase serum estradiol levels (Cook et al., 1994). These endocrine changes correlate with its potency to induce LCTs in rats and were hypothesized to play a role in the PFOA-induction of LCTs (Biegel et al., 2001). Other PPARα agonists (e.g., DEHP, clofibrate) have been shown to increase serum estradiol levels in adult male rats (Eagon et al., 1994; Rao et al., 1994). Eight out of eleven other peroxisome proliferators have been shown to increase estradiol production using isolated LCs (Liu et al., 1996). Collectively, these data suggest that many PPARα agonists can increase estradiol levels. It was postulated that the elevated estradiol levels may cause Leydig cell hyperpalsia and tumor formation by acting as a mitogen and/or enhancing growth factor secretion; the transforming growth factor α (TGF α), which binds to the epidermal growth factor (EGF) receptor and stimulated cell proliferation, for instance, has been detected in Leydig cell (Teerds et al., 1990).

Subsequent experiments have shown that PFOA increased the levels of estradiol by inducing CYP19 (aromatase), which converts testosterone to estradiol. Peroxisome proliferators are known to induce α-oxidation and cytochrome P-450 monooxygenases by binding to the peroxisome proliferation activation receptor α (PPAR α; a subfamily of steroid hormone receptors). It is possible that PFOA induces cytochrome P450 XIX (aromatase) by binding to and activating the PPAR α.

PFOA has also been shown to directly inhibit testosterone production when incubated with isolated LCs, while ex vivo studies demonstrated that this inhibition was reversible (Biegel et al., 1995). This inhibition of testosterone biosynthesis appears to be mediated by PPARα (Gazouli et

al., 2002) and may contribute to the development of LCT through disruption of the hypothalamus pituitary thyroid axis. Testosterone, which is synthesized and secreted by the Leydig cells, is regulated by LH; testosterone and LH form a closed-loop feedback system in the HPT axis. In order to maintain adequate testosterone plasma levels, reduced testosterone levels is expected to lead to increased LH levels through the negative feedback mechanism. In a mechanistic bioassay with PFOA, serum testosterone and LH levels were not significantly altered at the levels of PFOA that were tested (Biegel et al., 2001). It has been pointed out that increases in LH may not always be seen in all studies of chemicals for which the proposed mode of action calls for elevated LH, and that compensation may have occurred to restore homeostasis and inappropriate timing of sampling are some of the explanations for failing to detect changes in LH levels (Clegg et al., 1997).

Thus, the above data demonstrate that the induction of LCTs by PFOA may be attributed to a hormonal mechanism whereby PFOA either inhibits testosterone biosynthesis and/or increases serum estradiol levels via induction of hepatic aromatase activity. A critical review of these mode of action data by Klaunig et al. (2003) led to the conclusion that the evidence is inadequate at this time to support a linkage between PPARα agonism and induction of LCT. A similar conclusion was made by the FIFRA SAP (FIFRA SAP, 2004). The Panel recognized that only limited evidence support a link between Leydig cell tumors induction and a PPARα agonist mode of action, the SAP concurred with the conclusion that chemicals in this subclass that induce Leydig cell tumors may pose a carcinogenic hazard for humans.

Although the LCT induced in the rat by PFOA are assumed to be relevant to humans, they probably do not represent a significant cancer hazard for humans. Testicular tumors comprise about 1% of all human male neoplasms. LCT are rare as they account for only 1-3% of all testicular tumors in man (cited in: Prentice and Meikle, 1995). As there is some evidence that PFOA induced LCT in rats either by inhibiting testosterone biosynthesis and/or by inducing aromatase and thereby increasing estradiol levels, both the rat and human hypothalamic-pituitary-testicular axis may respond to inhibition of testosterone with a subsequent increase in LH. Hence, if PFOA inhibited testosterone biosynthesis in humans an increase in LH is expected to occur. However, a 6-month cynomolgus monkey study with PFOA did not demonstrate any compound-related effects on testosterone or estradiol (Butenhoff et al., 2002). These data suggest that PFOA is unlikely to induce LCT in humans because humans are quantitatively less sensitive than rats to LH stimulation (Cook et al., 1999). The number of LH receptors per Leydig cell is approximately 1,500 in man and 20,000 in the rat. Furthermore, the rat appears to be unique in possessing the Leydig cell luteinising hormone releasing hormone (LHRH) receptors which have the same effects on Leydig cells and are not present in man, monkey or mouse (cited in: Prentice and Meikle, 1995). The more compelling evidence supporting that testosterone inhibition or estradiol induction is unlikely to pose a significant cancer hazard/risk to humans may be the human disease state FMPP where men have a mutated LH receptor that is activated throughout their life and LCT are not seen (Cook et al., 1999).

3.9.3.4 Pancreatic Acinar Cell Tumors in Rats

The mechanism by which PFOA induced pancreatic acinar cell tumors is unknown. A number of other peroxisome proliferators also produce pancreatic acinar cell tumors in rats. The development of pancreatic acinar cell hypertrophy, hyperplasia, and adenomas in the rat have been shown to be modified by several factors such as steroids (testosterone and estradiol), growth factors such as cholecystokinin (CCK), growth factor receptor over-expression (CCK_A receptor) and diet (fat) (Longnecker, 1983; Longnecker, 1987; Longnecker and Sumi, 1990). These

potential mechanisms have been investigated in a series of in vitro and in vivo (subacute, subchronic, and oncogenicity) studies using PFOA (Obourn et al., 1997a; Obourn et al., 1997b). The available data suggest that PFOA appeared to induce PACTs by an indirect trypsin-inhibition mechanism where reduced bile flow and/or changes in bile composition produced an increase in CCK levels secondary to hepatic cholestasis; a sustained increase in CCK levels is responsible for the development of PACTs in rats (Cook et al., 1994; Obourn et al., 1997). CCK is a growth factor that has been shown to stimulate normal, adaptive, and neoplastic growth of pancreatic acinar cells in rats (Longnecker, 1987). Accordingly, the PACT induced in rats by PFOA (and other PPARα agonists) may be secondary to the liver effects of PPARα agonism. However, the mechanism(s) by which PFOA may induce PACT is based primarily on data with WY14643, a more potent pancreatic acinar cells carcinogen.

The available MOA data of PFOA and other PPARα agonists which induced PACT in rats was recently reviewed by Klaunig et al. (2003), and it was concluded that the evidence is inadequate at this time to support a linkage between PPARα agonism and induction of PACT. A similar conclusion was made by the FIFRA SAP (FIFRA SAP, 2004). The SAP concurred that chemicals in this subclass that induce pancreatic acinar cell tumors may pose a carcinogenic hazard for humans.

While there are some evidence that a sustained increase in CCK levels is responsible for the development of PACTs in rats by PFOA, expressions of PPARα and CCK_A receptors in humans are much lower than rodents. In addition, humans regulate pancreas exocrine secretion via a neuronal pathway rather than direct binding of CCK to acinar CCK_A receptors as in rodents. Consistent with this conclusion, a 6-month cynomolgus monkey study with PFOA did not demonstrate any compound-related effects on CCK levels or clinical pathology evidence of cholestasis (Butenhoff et al., 2002). Study of CCK levels in employees also did not report increases in workers (Olsen, et al., 1998b; Olsen et al., 2000). Furthermore, while the pancreatic tumors in the rat are typically derived from acinar cells, the majority of human pancreatic neoplasms are of the ductal type (Cotran et al.,1989). Therefore, the PACT induced in the rat by PFOA probably do not represent a significant cancer hazard for humans.

3.9.3.5 Cancer Descriptor

Carcinogenicity studies in Sprague-Dawley (CD) rats show that PFOA induces a "tumor triad" similar to a number of other PPARα agonists. This "tumor triad" includes liver tumors, Leydig cell tumors, and pancreatic acinar cell tumors. The evidence for mammary fibroadenomas in the female rats is equivocal since the incidences were comparable to some historical background incidences. In humans, occupational studies of still relatively young cohorts have not indicated statistically significant increases in these types of cancer. Not only were the numbers of cancer deaths very small in some of the cancer categories, but PFOA exposures were also not adequately characterized.

As summarized in section 3.9.3.1, there is sufficient evidence to conclude that PFOA is a PPARα-agonist and that PFOA induces liver toxicity and adenomas via a PPARα agonist MOA in rats. PFOA activates the PPARα and the requisite dose-response and/or temporal associations of the key events for the PPARα mode of action with the liver adenomas have been characterized. A recent ILSI workgroup as well as the FIFRA SAP have concluded that this MOA is unlikely to occur in humans based on quantitative differences in several of the key factors (Klaunig et al., 2003; OPPTS, 2003; FIFRA SAP, 2004).

The modes of carcinogenic action of PFOA-induced LCTs and PACT have not been fully elucidated. The induction of LCT by PFOA may involve a hormonal mechanism whereby PFOA either inhibits testosterone biosynthesis and/or increases serum estradiol levels via induction of hepatic aromatase activity. The induction of PACT are related to an increase in serum level of the growth factor, CCK, that appears to be secondary to changes in the liver. As the modes of carcinogenic action of PFOA-induced LCT and PACT have not been clearly linked to PPARα agonism, it is assumed that these tumors induced in rats are relevant to humans. However, the LCT and PACT induced in the rat by PFOA probably do not represent a significant cancer hazard for humans because of quantitative differences in the expressions of LH and CCK_A receptors and of other toxicodynamic differences between the rat and the human. For instance, the number of LH receptors per Leydig cell is approximately 1,500 in man and 20,000 in the rat. Furthermore, the rat appears to be unique in possessing the Leydig cell luteinising hormone releasing hormone (LHRH) receptors which have the same effects on Leydig cells and are not present in man, monkey or mouse (cited in: Prentice and Meikle, 1995). While there is some evidence that a sustained increase in CCK levels is responsible for the development of PACTs in rats by PFOA, expressions of PPARα and CCK_A receptors in humans are much lower than rodents. In addition, humans regulate pancreas exocrine secretion via a neuronal pathway rather than direct binding of CCK to acinar CCK_A receptors as in rodents; the pancreatic tumors in the rat are typically derived from acinar cells whereas the majority of human pancreatic neoplasms are of the ductal type (Cotran et al.,1989).

Overall, based on no adequate human studies and uncertain human relevance of the tumor triad (liver, Leydig cell and pancreatic acinar cell tumors) from the rat studies, PFOA may be best described as "*suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*"under the draft 1999 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999).

3.9.4 Toxicity in Adult Repeat-Dose Animal Studies

3.9.4.1 Non-Human Primates

A summary of the toxicity associated with exposure to APFO in adult non-human primates is presented in Table 21. In both the Rhesus and cynomolgus monkey studies, there was a very steep dose-response curve for mortality. The cause of the mortality is unknown. The Rhesus monkeys also exhibited several clinical indicative of gastrointestinal distress; the mode of action for these effects is unknown, but the gastrointestinal effects may be due to the potent surface activity properties of APFO. The gastrointestinal effects were not noted in a 6 month study of cynomolgus monkeys. The lack of gastrointestinal effects may be due to a species difference, or due to the fact that the monkeys were dosed via a capsule versus gavage in the Rhesus monkey study. In addition, liver weights were significantly increased in the cynomolgus monkey, but were unaffected in the Rhesus monkey. The increased liver weight does not appear to be due to peroxisome proliferation as there was only a minimal increase in palmitoyl CoA oxidase in the high dose group. However, there was evidence of mitochondrial proliferation which also suggests that a mode of action other than PPARα-agonism is operative in this species. It should also be noted that a number of hormonal and cell proliferation assays were included in the cynomolgus monkey study to determine whether there was any indication of a response of hepatic, Leydig cells and pancreatic acinar cells to PFOA exposure which have been observed in the rat. There was no indication of cell proliferation in the liver, testes or pancreas, nor were hormonal measures affected. There is significant uncertainty associated with the derivation of a LOAEL and NOAEL for both of these studies due to the small sample size, the variability in

response of the individual monkeys, and the lack of linear proportionality between administered dose and serum PFOA levels.

Table 21
Summary of Toxicity in Nonhuman Primates

Monkey	Exposure Period	Dose	Response	LOAEL/ NOAEL (mg/kg-day)	Reference
Rhesus 2/sex/dose	13 weeks	gavage 0, 3, 10, 30, 100 mg/kg-day	≥ 3 - clinical signs (including G.I) ≥ 30 - mortality, ↓ body weight, clinical signs	LOAEL = 3 no NOAEL	Goldenthal, 1978b
Cynomolgus 4-6 males/dose	6 months	oral capsule 0, 3, 10, 20/30 mg/kg-day	3 - moribund (sacrificed), clinical signs ≥ 3 - ↑absolute and relative liver weight 20/30 - moribund (sacrificed), clinical signs, treatment stopped, ↓body weight,	LOAEL = 3 NOAEL = ND	Thomford, 2001b; Butenhoff et al., 2002

3.9.4.2 Adult Male Rats

A summary of the systemic toxicity associated with APFO exposure in adult male rats is presented in Table 22. The major target organ is the liver. As described in section 3.9.2.1, the liver toxicity is due to a PPARα-agonist mode of action which is unlikely to occur in humans. Aside from the liver toxicity, APFO exposure is not associated with many other effects. Body weight was reduced at doses of 6.5 mg/kg-day in a 90-day study (Palazzolo, 1993); at ≥ 3 mg/kg-day in the F0 males and ≥ 1 mg/kg-day in the F1 males in the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004); and at 14.2 mg/kg-day in the 2-year study (Sibinski, 1987). Adrenal hypertrophy and vacuolation was noted in the F0 adult males at doses ≥ 10 mg/kg-day and in F1 adult males at 30 mg/kg-day in the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004). There is some uncertainty as to the biological significance of this finding since this effect was not observed in a 90-day study (Goldenthal, 1978a) or a 2-year study (Sibinski, 1987). Exposure to APFO was associated with a reduction in the number of erythrocytes and related parameters following exposure to 14.2 mg/kg-day APFO in the 2-year study (Sibinski, 1987). The testicular masses and testicular vascular mineralization observed in the 2-year study are probably associated with the etiology of the Leydig cell adenomas that were discussed in section 3.9.4.3. The mode of action is unknown for any of these effects.

Table 22
Summary of Systemic Toxicity in Adult Male Rats

Rat Strain	Exposure Period	Dose	Effects (mg/kg-day)	LOAEL/ NOAEL (mg/kg-day)	Reference
CD	13 weeks	diet - 0, 10, 30, 100, 300, 1000 ppm 0.056, 1.72, 5.64, 17.9, 63.5 mg/kg-day	≥ 1.72 - ↑absolute liver weight ≥ 5.64 - ↑hepatocellular hypertrophy ≥ 17.9 - ↑relative liver weight ≥ 63.5 - ↓body weight	LOAEL = 1.72 NOAEL = 0.56	Goldenthal, 1978a
Sprague-Dawley	13 weeks	diet - 0, 1, 10, 30, 100 ppm 0.06, 0.64, 1.94, 6.50 mg/kg-day	≥ 0.64 - ↑absolute and relative liver weight, hepatocellular hypertrophy, ↑palmitoyl CoA oxidase 6.5 - ↓body weight	LOAEL = 0.64 NOAEL = 0.06	Palazzolo, 1993
Sprague-Dawley	F0 animals ~15 weeks F1 animals ~19-20 weeks	gavage - 0, 1, 3, 10, 30 mg/kg-day	≥ 1 - ↑absolute and relative liver ≥ 3 - ↓body weight ≥ 10 - adrenal hypertrophy and vacuolation 30 - clinical signs, 1/30 moribund ≥1 - ↑absolute and relative liver weight, ↓body weight ≥3 - hepatocellular hypertrophy 30 - adrenal hypertrophy and vacuolation	LOAEL =1 no NOAEL LOAEL =1 no NOAEL	York, 2002; Butenhoff et al., 2004
Sprague-Dawley	2 years	diet - 0, 30, 300 ppm 1.3, 14.2 mg/kg-day	14.2 - ↑relative liver weight, hepatocellular hypertrophy, cystoid degeneration, ALT; ↓ body weight gain; ↓erythrocytes, hematocrit, hemoglobin concentration; testicular vascular mineralization, testicular masses	LOAEL = 14.2 NOAEL = 1.3	Sibinski, 1987
Sprague-Dawley	2 years	diet - 0, 300 ppm 14.2 mg/kg-day	14.2 - ↑ relative liver weight	LOAEL = 14.2 no NOAEL	Cook et al., 1994; Biegel et al., 2001

3.9.4.3 Adult Female Rats

A summary of the systemic toxicity associated with APFO exposure in the adult female rat is shown in Table 23. Due to the gender difference in elimination of PFOA in rats, the response of the female rat to APFO exposure is generally far less than that of the male rat. The effects on the liver were only observed at relatively high doses (Goldenthal, 1978a). The effects on the adrenal that were observed in the male rat were not observed in the female rat in the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004). Body weight was reduced at doses of 30 mg/kg-day in the F1 females in the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004), and at 16.1 mg/kg-day in the 2-year study (Sibinski, 1987). Exposure to APFO was associated with a reduction in the number of erythrocytes and related parameters following exposure to 16.1 mg/kg-day APFO in the 2-year study (Sibinski, 1987). The mode of action is unknown for any of these effects.

Table 23
Summary of Systemic Toxicity in Adult Female Rats

Rat Strain	Exposure Period	Dose	Effects (mg/kg-day)	LOAEL/ NOAEL (mg/kg-day)	Reference
CD	13 weeks	diet 0, 10, 30, 100, 300, 1000 ppm 0.74, 2.3, 7.7, 22.4, 76.5 mg/kg-day	76.5 - ↑absolute and relative liver weight	LOAEL = 76.5 NOAEL = 22.4	Goldenthal, 1978a
Sprague-Dawley	F0 animals ~18 weeks F1 animals ~18 weeks	gavage 0, 1, 3, 10, 30 mg/kg-day	No effects 30 - ↓ body weight	no LOAEL NOAEL = 30 LOAEL = 30 NOAEL = 10	York, 2002; Butenhoff et al., 2004
Sprague-Dawley	2 years	diet 0, 30, 300 ppm 1.6, 16.1 mg/kg-day	16.1 - ↓body weight gain, ↓erythrocytes, hematocrit, hemoglobin concentration	LOAEL = 16.1 NOAEL = 1.6	Sibinski, 1987

3.9.4.4 Adult Mice

In mice, a 28 day study demonstrated that the liver is also a target organ. As described above (section 3.9.3), the liver toxicity is due to a PPARα-agonist mode of action which is unlikely to occur in humans. In addition, several studies have shown that PFOA affects the immune system in mice. Feeding C57Bl/6 mice a diet containing 0.02% PFOA resulted in adverse effects to both the thymus and spleen (Yang et al. 2000, 2001). In addition, this feeding regimen resulted in

suppression of the specific humoral immune response to horse red blood cells, and suppression of splenic lymphocyte proliferation in response to LPS and ConA (Yang et al., 2002b). The suppressed mice recovered their ability to generate a humoral immune response when they were fed a diet devoid of PFOA. Studies using transgenic mice showed that the PPARα was involved in causing the adverse effects to the immune system (Yang et al., 2002a). Further research is needed to determine the exact role of PPARα and immunotoxicity in mice.

3.9.5 Developmental and Reproductive Toxicity in Animal Studies

The developmental and reproductive effects associated with oral exposure to APFO in animal studies are summarized in Table 24. In New Zealand White rabbits, there was evidence of prenatal developmental toxicity following exposure to 50 mg/kg-day APFO on gestation days 6-18 (Gortner, 1982). In contrast, there was no evidence of developmental toxicity following exposure to doses as high as 150 mg/kg-day APFO during gestation days 6-15 in Sprague-Dawley rats (Gortner, 1981; Staples, 1984). However, there was evidence of developmental/reproductive toxicity during the postnatal period in a two generation reproductive toxicity study in Sprague-Dawley rats (York, 2002; Butenhoff et al., 2004). In this study, During lactation, there was a reduction in F1 mean body weight on a litter basis during lactation (sexes combined) in the 30 mg/kg-day group. F1 males in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. F1 females in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 males and females in the 30 mg/kg-day group. For F1 males, the LOAEL for developmental/reproductive toxicity was 10 mg/kg-day, and the NOAEL was 3 mg/kg-day. For F1 females, the LOAEL for developmental/reproductive toxicity was considered to be 30 mg/kg-day, and the NOAEL was 10 mg/kg-day. No effects were observed in the F2 pups. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation.

Table 24
Summary of Developmental and Reproductive Toxicity Effects

Species	Dose (mg/kg-day)	Exposure Duration	Time Assessed	Effects	LOAEL/ NOAEL	Reference
New Zealand White rabbit	0, 1.5, 5, 50	GD 6-18	GD 29	↑13th rib	LOAEL = 50 NOAEL = 5	Gortner, 1982
Sprague-Dawley rat	0, 0.05, 1.5, 5, 150	GD 6-15	GD 20	none	no LOAEL NOAEL = 150	Gortner, 1981
Sprague-Dawley rat	0, 100	GD 6-15	GD 20	none	no LOAEL NOAEL = 100	Staples, 1984
Sprague-Dawley rat	0, 100	GD 6-15	PND 35	none	no LOAEL NOAEL = 100	Staples, 1984
Sprague-Dawley rat	0, 1, 3, 10, 30	F1 - prior to conception through sexual maturation F2 - prior to conception until weaning	Variety Variety	≥ 10 - ↓postweaning body weight in males 30 - ↓preweaning litter body weight , ↑postweaning mortality, delayed sexual maturation, ↓postweaning body weight in females none	LOAEL = 10 NOAEL = 3 no LOAEL NOAEL = 30	York, 2002; Butenhoff et al., 2004

4.0 Biomonitoring Data

PFOA has been measured in human serum of workers occupationally exposed to APFO and in the general U.S. population. In general, serum PFOA concentrations in the general population are much lower than in workers exposed to APFO (see Tables 25-27 below). However, it should be noted that the highest levels reported to date in the general population are similar to some of the lowest levels in workers exposed to PFOA occupationally. The environmental concentrations of APFO and the pathways of exposure to the general population are not known.

4.1 Occupational Exposures

3M and DuPont have been the primary producers and users of perfluorinated compounds in the U.S. Both companies offer voluntary medical surveillance programs to workers at plants that produce or use perfluorinated compounds. 3M discontinued manufacturing PFOA between 2000-2002. PFOA serum levels seem to be decreasing slightly at both the 3M and DuPont facilities; however, the cross-sectional nature of the data cannot provide trends in serum concentrations. PFOA blood serum data for volunteers in the medical surveillance programs at these plants that have been submitted to EPA are presented below in μg/mL (ppm).

4.1.1 3M Occupational Data

3M has been offering voluntary medical surveillance to workers at plants that produce or use perfluorinated compounds since 1976. Serum PFOA levels in 3M workers have been measured since 1993 (Olsen et al., 2003a; 2003b; 2003c; 2001a; 2001b; 2001c; 2000; 1999; 1998c). Prior to this time, analytical capabilities precluded the accurate measurement of any specific fluorochemical analyte and only total organic fluorine was measured. PFOA analysis differed slightly in each surveillance program year and different laboratories were used to assay PFOA in each year. The samples were analyzed for PFOA using high performance liquid chromatography mass spectrometry (HPLC/MS), but the extraction methods differed slightly each year (Olsen, et al., 2001f; Olsen, et al., 2003a; 2003b).

Serum PFOA concentrations for workers participating in 3M's biomonitoring program have been reported for 3 plants: Cottage Grove, Minnesota; Decatur, Alabama;and Antwerp, Belgium. Only the U.S. data are presented here. Surveillance years included 1993, 1995, 1997, 1998, 2000, 2002, and 2003, although not all of the plants offered surveillance in all of these years. Participation in all of the program years was voluntary. The eligible voluntary participation rates ranged from approximately 70% in 1993 to 50% in 1997 (Olsen, et al., 2001f). The number of employees volunteering for participation increased greatly in 2000 and then declined by about 75% in 2002 (Olsen, et al., 2003a; 2003b) (see Table 24 below). 3M suggests that the increase in 2000 was probably due to increased employee awareness of the persistence and prevalence of perfluorinated chemicals in human tissues. Meanwhile, the decline in 2002 was probably due to 3M's announcement to phase out PFOA production at their plants. In 1998, a random sample of Decatur employees was surveyed to determine whether the voluntary nature of the program was biasing the results of the surveillance (Olsen et al., 1999). The data presented in Table 24 indicate that there is little difference in male serum concentrations between 1997 (1.40 μg/mL, when participation was voluntary) and 1998 (1.735 μg/mL, when a random sample was taken).

Gender-based data for 3M's biomonitoring program are limited due to the small number of women participating in the program. Mean PFOA serum concentrations for female employees at

all 3 of the plants were lower than those of male employees, even in similar jobs. Where data are available by gender, they are provided in Table 25.

Table 25
Summary of 3M U.S. Occupational PFOA Biomonitoring
(serum concentrations, μg/mL)*

Plant	Arith-metic Mean	Range	Geometric Mean	95% CI of Geometric Mean	Reference
Cottage Grove					
2002 (n=38)	4.3	0.07 - 32.6	1.7	1.02-2.72	Olsen, et al., 2003a
2000 (n = 148)	NR	NR	NR	NR	Olsen, et al., 2003f
Male (n = 131)	4.51	.007-92.03	0.85	0.64- 1.22	
Female (n = 17)	0.85	.04 - 4.73	0.42	0.23 - 0.79	
1997 (n =74)	6.4	0.1 - 81.3	NR	NR	Olsen, et al., 2000
1995 (n = 80)	6.8	0 - 114.1	NR	NR	Olsen, et al., 2000
1993 (n = 111)	5.0	0 - 80.0	NR	NR	Olsen, et al., 2000
Decatur					
2002 (n =54)	1.497	0.025-4.81	0.713	0.483-1.055	Olsen, et al., 2003b
2000 (n = 263)	1.78	0.04-12.70	1.13	0.99 - 1.30	Olsen, et al., 2001a
Male (n = 215)	1.90	0.04-12.70	NR	NR	Olsen et al., 2003e
Female (n = 48)	1.23	NR	NR	NR	Olsen et al., 2003e
1998 (n = 126)	1.54	0.02 - 6.76	0.90	0.72 -1.12	Olsen, et al., 1999
Male (n =102)	1.735	0.02 - 6.76	1.142	NR	
Female (n=24)	0.691	NR	0.326	NR	
1997 (n = 84) (males only)	1.40	NR	NR	NR	Olsen, et al., 1998c
1995 (n = 90) (males only)	1.72	NR	NR	NR	Olsen, et al., 1998c
Building 236 (fluorochemical research) 2000 (n = 45)	0.106	0.008-0.668	0.053	0.037 -0.076	Olsen, et al., 2001c

*μg/mL = ppm
NR- not reported

Of the plants listed above, PFOA exposures are highest at the Cottage Grove plant. Correspondingly, PFOA serum levels are highest at this plant (geometric mean = 1.7 μg/mL, range, 0.07 - 32.6 μg/mL). Mean serum PFOA levels increased slightly at both the Cottage Grove and Decatur plants from 1993 to 2000; however, the latest serum measurements (2002) show a slight decline in mean PFOA concentrations. However, it should be noted that the number of participants in the 2002 sampling period was comparatively small. In addition, all of the data are cross-sectional and therefore cannot provide any temporal information.

4.1.2 DuPont Occupational Data

Dupont has been measuring PFOA in workers' blood serum since 1981 (Dupont, 2001a, 2001b). Prior to 1981, total blood fluoride levels were reported. All of Dupont's operations in the U.S. that use PFOA with significant exposure potential are concentrated at the Washington Works plant in Washington, West Virginia. PFOA is used as a fluoropolymer reaction aid at this plant. The data presented in Table 26 reflect serum concentrations of volunteer workers in the plant who had potential PFOA exposure. With the exception of the 2000 sampling period, the same workers were common to all of the other sampling periods, with additional workers participating in 1995. The data available to EPA at this time are limited to what is presented here.

Table 26
Summary of Dupont Occupational PFOA Biomonitoring
(serum concentrations, μg/mL)*

Year	Arithmetic Mean	Range	Reference
Washington Works			
2000 (n= 72)	1.53	0.02 - 9.0	Dupont, 2001a
1995 (n = 80)	1.56	0.12 - 4.5	Dupont, 2001b
1989-90 (n =22)	1.96	0.06 - 11	Dupont, 2001b
1985 (n = 22)	2.34	0.06 - 18	Dupont, 2001b
1984 (n = 19)	3.21	0.07 - 24	Dupont, 2001b

*μg/mL = ppm

4.2 General Population Exposures

Data on PFOA levels in the general population include both pooled and individual serum samples. Mean serum PFOA levels are lower in the general population than in workers exposed to PFOA (described above). Individual serum PFOA levels in three separate US cohorts are very similar. The geometric mean for all three cohorts, comprised of 3 separate age groups, is approximately 0.004 μg/mL (4 ppb). All of the data are fairly recent but are cross-sectional; therefore, temporal trends cannot be established. The available data are summarized in Table 27.

Table 27
Summary of General Population PFOA Biomonitoring
(serum concentration, μg/mL[a])

Sample	Arithmetic Mean	Range	Geometric Mean	95% CI	Reference
Pooled Samples					
Commerical sources of blood, 1999 (n = 35 lots)	0.003	.001 - .013	NA	NA	3M Company, 1999a
Blood banks, 1998 (n = 18 lots, 340-680 donors)	0.017[b]	0.012 - 0.022	NA	NA	3M Company, 1999b
Individual Samples					
American Red Cross blood banks, 2000 (n = 645)	0.0056	0.0019 - 0.0523	0.0046	0.0043 - 0.0048	Olsen et al., 2002a, 2003d
Elderly (65 - 96 years), 2000 (n = 238)	NR	0.0014 - 0.0167	0.0042	0.0039 - 0.0045	Olsen et al., 2002b, 2004a
Children (2 - 12 years), 1995 (n = 598)	0.0056	0.0019 - 0.0561	0.0049	0.0047 - 0.0051	Olsen et al., 2002c, 2004b

[a] μg/mL = ppm
[b] PFOA detected in about 1/3 of the pooled samples but quantifiable in only 2.
NR - not reported

Pooled blood samples from U.S. blood banks indicate mean PFOA levels of 0.003 to 0.017 μg/mL (3 - 17 ppb) (3M Company, Feb. 5, 1999; 3M Company, May 26, 1999). The highest pooled sample reported was 0.022 μg/mL (22 ppb). Pooled samples were collected in 1998 and 1999. However, it cannot be assumed that these levels can be generalized to the U.S. population for several reasons: 1) blood donors are a unique group that does not necessarily reflect the U.S. population as a whole, 2) many of the blood banks originally contacted for possible inclusion in the study declined to participate, 3) only a small number of samples have actually been analyzed for PFOA, and 4) no other data such as age, sex, or other demographic information are available on the donors.

Individual blood samples from 3 different age populations were recently analyzed for PFOA and other fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMS) (Olsen et al., 2002a, 2002b, 2002c). The studies' participants included adult blood donors, an elderly population participating in a prospective study in Seattle,

WA, and children from 23 states participating in a clinical trial. Overall, the PFOA geometric means were similar across all 3 populations (0.0046 µg/mL, 0.0042 µg/mL, and 0.0049 µg/mL, respectively). The geometric means and 95% tolerance limits (the exposure below which 95% of the population is expected to be found) and their upper bounds were comparable across all 3 studies. However, the upper ranges for the children and adults were much higher than for the elderly population. It is not clear whether this is the result of geographic differences in PFOA levels or some other factor. It should be noted that PFOS and PFOA were highly correlated in all three studies ($r = .63$, $r = .70$, and $r = .75$) and that PFOA did not meet the criteria for a log normal distribution based on the Shapiro-Wilk test in any of the studies. The authors suggest that it may be due to the greater proportion of subjects with values less than the lower limit of quantitation (LLOQ); however, only 12 of the 1481 total samples were below the LLOQ. In those instances where a sample was measured below the LLOQ, the midpoint between zero and the LLOQ was used for calculation of the geometric mean. The details of each study are provided below.

Blood samples from 645 U.S. adult blood donors (332 males, 313 females), ages 20-69, were obtained from six American Red Cross blood banks located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD (Olsen et al., 2002a). Each blood bank was requested to provide approximately 10 samples per 10-year age intervals (20-29, 30-39, etc.) for each sex. The only demographic factors known for each donor were age, gender, and location.

The geometric mean serum PFOA level was 0.0046 µg/mL. The range was <lower limit of quantitation (0.0019 µg/mL) to 0.0523 µg/mL. Only 2 samples were less than the LLOQ. Males had significantly higher ($p < .05$) geometric mean PFOA levels than females (0.0049 µg/mL vs. 0.0042 µg/mL). Age was not an important predictor of adult serum fluorochemical concentrations. When stratified by geographic location, the highest geometric mean for PFOA was in the samples from Charlotte, NC (0.0063 µg/mL, range: 0.0021 – 0.029 µg/mL) and the lowest from Portland (0.0036 µg/mL, range: 0.0021 – 0.0167 µg/mL). The highest individual value was reported in Hagerstown (0.0523 µg/mL).

Serum PFOA levels were reported for 238 (118 males and 120 females) elderly volunteers in Seattle participating in a study designed to examine cognitive function in adults aged 65-96 (Olsen et al., 2002b, 2004a). Age, gender and number of years' residence in Seattle were the only data available on the participants. Most of the participants were under the age of 85 and had lived in the Seattle area for over 50 years.

The geometric mean of PFOA for all samples was 0.0042 µg/mL. The range was 0.0014 – 0.0167 µg/mL. Only 5 samples were less than the LLOQ of 0.0014 µg/mL. There was no significant ($p < .05$) difference in geometric means for males and females. In simple linear regression analyses, age was negatively ($p < .05$) associated with PFOA in elderly men and women. PFOS and PFOA were highly correlated ($r = .75$) in this study.

A sample of 598 children, ages 2-12 years old, participating in a study of group A streptococcal infections, was analyzed for serum PFOA levels (Olsen et al., 2002c, 2004b). The samples were collected in 1994-1995 from children residing in 23 states and the District of Columbia. PFOA did not meet the criteria for a log normal distribution based on the Shapiro-Wilk test. The authors suggest that it may be due to the greater proportion of subjects with values < LLOQ for PFOA; however, only 5 samples were less than the LLOQ of 0.0019 µg/mL. The geometric mean of PFOA for all of the participants was 0.0049 µg/mL, and the range was 0.0019 to 0.0561 µg/mL.

Male children had significantly ($p<.01$) higher geometric mean serum PFOA levels than females: 0.0052 µg/mL and 0.0047 µg/mL, respectively. In simple linear regression analyses, age was significantly ($p < .05$) negatively associated with PFOA in both males and females. When stratified by age, the geometric mean of PFOA was highest at age 4 (0.0057 µg/mL) and lowest at age 12 (0.0035 µg/mL). Although the data were not reported, a graphical presentation of log PFOA levels for each state by gender looked similar across the states. However, it is difficult to interpret these data without analyzing them and the sample sizes were limited for each gender/location subgroup. PFOS and PFOA were highly correlated ($r = .70$) in this study. PFOA and PFHS (perfluorohexanesulfonate) were also correlated, although not as strongly ($r = .48$).

The above 3 studies indicate similar geometric means and ranges of PFOA among sampled adults, children, and an elderly population. However, an unexpected finding was the level of PFHS and M570 (N-methyl perfluorooctanesulfonamidoacetate) in children (Olsen, 2002c, 2004b). These serum levels were much higher in the sampled children than in the sampled adults or elderly. It is not clear why this occurred, but it is probably due to a different exposure pattern in children.

In another study, the PFOA concentration was analyzed in human sera and liver samples using HPLC/ESMS (Olsen et al., 2001d). Thirty-one donor samples were obtained from 16 males and 15 females. The average age of the male donors was 50 years (SD 15.6, range 5-69) and the average age of the female donors was 45 years (SD 18.5, range 13-74). The causes of death were intracranial hemorrhage (n = 16 or 52%), motor vehicle accident (n = 7 or 23%), head trauma (n = 4 or 13%), brain tumor (n = 2 or 6%), drug overdose (n = 1 or 3%) and respiratory arrest (n = 1 or 3%). Both serum and liver tissue were obtained from 23 donors; 7 donors contributed liver tissue only and 1 donor contributed serum only. Resulting serum values for PFOA ranged from < LOQ (<0.0030) - 0.0070 µg/mL. Assuming the midpoint value between zero and LOQ serum value for samples <LOQ, the mean serum PFOA level was calculated as 0.0031 µg/mL with a geometric mean of 0.0025 µg/mL. No liver to serum ratios were provided because more than 90% of the individual liver samples were <LOQ.

5.0 Risk Assessment

A margin of exposure (MOE) approach can be used to describe the potential for human health effects associated with exposure to a chemical. The MOE is calculated as the ratio of the NOAEL or LOAEL for a specific endpoint to the estimated human exposure level. The MOE does not provide an estimate of population risk, but simply describes the relative "distance" between the exposure level and the NOAEL or LOAEL. In this risk assessment there is no information on the sources or pathways of human exposure. However, serum levels of PFOA, which are a measure of cumulative exposure, were available from human biomonitoring studies. In addition, serum levels of PFOA were available for many of the animal toxicology studies or there was sufficient pharmacokinetic information to estimate serum levels. Thus, in this assessment internal doses from animal and human studies were compared; this is somewhat analogous to a MOE approach which uses external exposure estimates.

5.1 Selection of Endpoints

The epidemiology and animal toxicology studies are summarized in section 3.4.9. The results of existing epidemiology studies are not adequate for use in quantitative risk assessment, and therefore the analysis is restricted to endpoints in the animal toxicology studies. A variety of studies have examined the potential toxicity of PFOA in adult animals.

Studies in mice have shown an effect on the immune system. These mouse studies were not considered for the calculation of the MOEs since information on serum levels of PFOA was not available, nor are there pharmacokinetic data that would allow for the estimation of serum levels.

As summarized in section 3.9.5.1, studies of non-human primates did not establish a NOAEL. The LOAEL for the 13-week study in Rhesus monkeys was 3 mg/kg-day based on clinical signs of toxicity (Goldenthal, 1978b) and the LOAEL for the 6-month study of cynomolgus monkeys was also 3 mg/kg-day based on increased liver weight and possibly mortality (Thomford, 2001b; Butenhoff et al., 2002). For calculation of MOEs, the cynomolgus monkey study was chosen since it included a slightly larger sample size (N = 4 to 6), was of longer duration, and included more robust serum analyses of PFOA.

Several studies have examined the toxicity of APFO in adult male and female rats which are summarized in sections 3.9.5.2 and 3.9.5.3, respectively. In adult male rats, the primary target organ is the liver. However, as described in section 3.9.3, the proposed mode of action for PFOA (PPARα-agonism) in rodents leading to the observed liver toxicity is unlikely to occur in humans, and therefore was not considered for calculation of the MOEs. The only other consistent effect noted across studies was body weight reduction. Body weight was reduced at doses of 6.5 mg/kg-day in a 90-day study (Palazzolo, 1993); at $\geq$ 3 mg/kg-day in the F0 males and $\geq$ 1 mg/kg-day in the F1 males in the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004); and at 14.2 mg/kg-day in the 2-year study (Sibinski, 1987). For adult female rats, there are very few effects due to the rapid excretion of PFOA. The only consistent effect that was noted among the studies was reduced body weight; this was observed at doses of 16.1 mg/kg-day in the 2-year study (Sibinski, 1987) and at 30 mg/kg-day in the adult F0 and F1 females in the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004). Although, the significance of reduced body weight for human health is not clear, body weight was used for the calculation of the MOEs simply because it defines the lowest effect level and therefore serves as a conservative benchmark. The lowest dose that was associated with reductions in body weight in males was in the F1 males in the two-generation reproductive toxicity study. For the F1 males, the LOAEL for body weight is 1 mg/kg-day, and a NOAEL was not established. The lowest dose for females was in the 2-year study; the LOAEL for body weight was 16.1 mg/kg-day and the NOAEL was 1.6 mg/kg-day.

Several studies have examined the developmental and reproductive toxicity associated with exposure to APFO which are summarized in section 3.9.5.5. Developmental effects were observed in a prenatal developmental toxicity study in New Zealand White rabbits (Gortner, 1982). However, this study was not considered for the calculation of the MOEs since information on serum levels of PFOA in rabbits was not available, nor are there pharmacokinetic data that would allow for the estimation of serum levels in rabbits. There were no developmental effects noted in prenatal developmental toxicity studies of Sprague-Dawley rats following exposure to APFO during gestation days 6-15 (Gortner, 1981; Staples, 1984). Developmental effects were noted in the F1 male and female pups in the two-generation reproductive toxicity study. During lactation, there was a reduction in F1 mean body weight on a litter basis (sexes combined) in the 30 mg/kg-day group. F1 males in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. F1 females in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation

for F1 males and females in the 30 mg/kg-day group. These endpoints were used for the calculation of the MOEs.

5.2 Use of Serum Levels as a Measure of Internal Dose for Humans

5.2.1 General Population

A summary of the human serum levels of PFOA that were considered in the calculation of MOEs is provided in Table 28. These data are derived from two biomonitoring studies performed on large cohorts from various geographic areas of the US and include both children and adults. They are described in detail in Section 4.2. The arithmetic and geometric means, as well as the range, 95% confidence intervals for the geometric means, and the cumulative 90th percentiles are displayed in order to provide a sense of the distribution of the data. The frequency distributions of the serum data appear to be log-normally distributed, although the criteria for the Shapiro-Wilk test are not met. This may be due to the number of subjects with serum values less than the LLOQ (n = 2 for <1.9 ng/ml and n = 48 for < 2.1 ng/ml). Gender specific data were available for the geometric mean and range, but not for the arithmetic mean. These serum values are assumed to represent steady state levels in the general US population.

Table 28
Summary of Levels of PFOA (μg/mL) in the Serum of Human Populations

Population	Arith-metic Mean	90th percentile	Range	Geo-metric Mean (GM)	95% CI GM
Adults (20 - 69 years, American Red Cross blood banks, 2000)					
Both genders (n = 645)	0.0056	0.0094	0.019-0.0523	0.0046	0.0043-0.0048
Males (n = 332)	NR	0.0101	<0.0019-0.029	0.0049	0.0046 - 0.0053
Females (n = 313)	NR	0.0084	<0.0021-0.0523	0.0042	0.0039 - 0.0045
Children (2-12-years, 1995)					
Both genders (n = 598)	0.0056	0.0085	0.0019 - 0.0561	0.0049	0.0047 - 0.0051
Males (n = 300)	0.0059	0.0090	<0.0029- 0.0561	0.0052	0.0049 - 0.0052
Females (n = 298)	0.0052	0.0080	<0.0019- 0.0186	0.0047	0.0044 - 0.0049

Unless stated otherwise, the geometric mean and 90th percentiles for the combined male and female adults and combined male and female children are used in the calculations of the MOEs in the following sections.

5.2.2 Workers

Although some serum level data were available for workers, the data were not adequate to calculate MOEs for specific occupational exposures. As described in section 4.1, 3M and DuPont have provided serum monitoring to workers on a voluntary basis. 3M discontinued manufacturing PFOA between 2000-2002. The last serum monitoring was offered at the 3M plants in 2002, but there were about 75-80% fewer volunteers in 2002 than in 2000. Thus, the sample size for 2002 is very small. In addition, since occupational exposures no longer exist for 3M workers, use of the blood monitoring data for 2000 may overestimate current serum levels.

The data available for the DuPont workers was very limited and there was no information available for a variety of critical factors including gender, sampling methods, and occupation.

In general, the mean serum levels following occupational exposures appear to be orders of magnitude higher than observed in the general population. Thus, MOEs for workers would be expected to be much less than for the general population.

5.3 Calculation of MOEs Based on Non-Human Primate Studies

As described in section 5.1, two studies have been conducted in non-human primates. A LOAEL of 3 mg/kg-day was observed in both studies, and neither study established a NOAEL. For calculation of MOEs, the 6-month male cynomolgus monkey study was chosen since it was a more robust study. The effects observed at 3 mg/kg-day included increased liver weight and possibly mortality. Serum levels of PFOA were measured throughout the study and a steady state level of 77 $\pm$ 39 μg/ml was observed in the 3 mg/kg-day group.

The most relevant general human population for use in the calculation of the MOEs is the population of adults, age 20-69, that was examined in the American Red Cross samples (Table 27). Although the cynomolgus monkey study was conducted in males, it should be noted that there is no evidence of a gender difference in the half-life of PFOA in humans or non-human primates. Comparing males and females, there was no evidence of a gender difference in toxicity in the Rhesus monkey study. In addition, the serum levels in the adult male and female human populations are very similar. Therefore, MOEs were calculated using the serum levels from the combined males and females. The MOEs were calculated by dividing the steady state serum value for the male cynomolgus monkeys in the 3 mg/kg-day group (77 μg/ml) by the adult serum levels from the American Red Cross blood samples presented in Table 28. The MOE calculated using the geometric mean (.0046 μg/ml) for the human serum level is 16,739. The MOE calculated using the 90th percentile value (.0094 μg/ml) for the human serum data is 8191.

5.4 Calculation of MOEs Based on Adult Rat Studies

As described in section 5.1, the effect levels that are appropriate for calculation of the MOEs are the LOAEL of 1 mg/kg-day for adult male rats from the two-generation reproductive toxicity study, and the NOAEL of 1.6 mg/kg-day for adult female rats from the 2-year study. Information on the serum levels of PFOA were not available for either study. Therefore, pharmacokinetic information was used to estimate the Area Under Concentration curve in plasma (AUC) as described in Appendix A. The estimated AUC for adult male rats exposed to 1 mg/kg-day is1011 μg-hr/mL and the estimated AUC for adult female rats exposed to 1.6 mg/kg-day is 44 μg-hr/mL.

The most relevant general human population for use in the calculation of the MOEs is the population of adults, age 20-69, that was examined in the American Red Cross samples (Table 28). Assuming that the serum levels reflect steady state in humans, an AUC was calculated by multiplying the serum concentrations by 24 hours. Thus, the AUC for the geometric mean is 0.1104 μg-hr/mL (0.0046 μg/mL X 24 hours), and the AUC for the 90th percentile is 0.2256 μg-hr/mL (0.0094 μg/mL X 24 hours). MOEs were calculated by dividing the AUC in the adult female rat by the AUC for the adult humans which is 398 (195 for the 90th percentile) and by dividing the AUC for the adult male rat by the AUC for the adult humans which is 9158 (4481 for the 90th percentile).

5.5 Calculation of MOEs Based on Rat Developmental Toxicity Studies

As described in section 5.1, developmental effects were observed in a two-generation reproductive toxicity study in Sprague-Dawley rats (York, 2002). These effects were observed at various times during the maturation of the F1 pups. During lactation, there was a reduction in F1 mean body weight on a litter basis (sexes combined) in the 30 mg/kg-day group. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 males and females in the 30 mg/kg-day group. Mean body weights were also significantly reduced prior to sexual maturation in the F1 males exposed to 10 and 30 mg/kg-day APFO and in the F1 females exposed to 30 mg/kg-day APFO.

The critical period of exposure for each of the effects is not known. For example, it is not known whether prenatal and/or lactational exposure is important for the reduced body weight that was observed during lactation. Similarly, it is not known whether the reduced body weight, mortality, or delayed sexual maturation that occurred during the postweaning period are due to prenatal, lactational, and/or postweaning exposures. Therefore, it is necessary to calculate MOEs for each of these exposure periods.

Only limited information on serum levels of PFOA were obtained in the two-generation reproductive toxicity study. This included measurements of serum levels in the F0 dams in the 10 and 30 mg/kg-day groups which were taken 24 hours after dosing. Given the rapid excretion of PFOA by female rats, these values are of limited utility. In addition, serum levels were not obtained for the F1 animals. Therefore, it was necessary to estimate serum levels based on pharmacokinetic information.

For the prenatal period, information was available on serum levels in the pregnant rat and fetus, as well as concentrations of PFOA in the embryo. However, the information on serum levels in the general U.S. population is restricted to children and adults; there is no information on levels in the human embryo or fetus. Therefore, it is only appropriate to derive MOEs based on serum levels in the pregnant rat and human females. Estimates of C_{max}, and AUC in the pregnant rat were used for calculation of the MOEs since it is not known which dose metric may be more significant. These dose metrics were estimated for a pregnant rat administered 3 mg/kg-day APFO since the lowest NOAEL for the developmental effects in the two-generation reproductive toxicity study was 3 mg/kg-day.

As described in section 3.2.4.1, PFOA serum levels were measured in the pregnant rat on gestation days 10, 15 and 21 following exposure to 3, 10, or 30 mg/kg-day APFO (Mylchreest, 2003). The serum levels were determined two hours after dosing. Kemper (2003) determined that peak serum concentrations of PFOA occur approximately 0.5 to 2 hrs after gavage dosing in the adult female rat. Thus, the serum levels obtained by Mylchreest (2003) are approximately equivalent to C_{max}. An average C_{max} for the pregnant rat was then calculated by averaging the serum levels obtained at gestation days 10, 15 and 21; this value is 13 μg/ml for the 3 mg/kg-day dose group. The AUC was estimated using a one compartment model which is described in Appendix A. The AUC for the pregnant rat administered 3 mg/kg-day is approximately 83 μg-hr/mL.

The most appropriate human population for comparison is the group of adult females, ages 20 - 69 years (Table 28). MOEs for the prenatal period were calculated by dividing the average C_{max} for the pregnant rat by the measured serum levels for adult human females which is 3,095 (1548 using the 90th percentile for human females), and by dividing the estimated AUC for the pregnant

rat by the AUC for adult human females which is 823 (412 using the 90th percentile for human females).

Estimation of meaningful serum levels in the F1 pups during lactation is problematic. As described in section 3.2.4.1, Mylchreest (2003) measured serum PFOA concentrations on days 3, 7, 14, and 21 of lactation. These measurements were taken approximately 2 hours after the dams were dosed. Pups were separated from the dams 1-2 hours prior to the time that blood samples were collected, and therefore it is not clear when the pups last nursed relative to the time that their blood samples were collected. Han (2003) has shown that the clearance in 4-week old male and female pups is fairly rapid. Assuming that the clearance is also rapid during the lactation period, steady state would not be achieved. Thus, the measured serum concentrations in the pups would not be expected to estimate steady state concentrations, and in fact, it is not clear what dose metric the serum levels represent. The AUC for the pups could also be estimated, but, this requires knowledge of milk consumption and elimination constants during lactation. While estimates of milk consumption and elimination constants could be made, such estimates would introduce considerable uncertainty in the resulting estimates of the AUC. Therefore, it is premature at this point to derive estimates of the AUC for the lactation period. In addition, there is no information from the available human biomonitoring studies on serum levels in children less than two years of age. Consequently, MOEs were not calculated for the lactation period.

MOEs were calculated for the postweaning period. As described in section 3.2.4.2, Han (2003) measured serum concentrations of PFOA in rat weanlings following a single dose of 10 mg/kg-day APFO at 4, 5, 6, 7 and 8 weeks of age. Using a one compartment model, this information was used to provide estimates of AUC (Appendix A). In the two-generation reproductive toxicity study, several effects were noted during the postweaning period. One of these effects was mortality that occurred mainly during the first few days after weaning in the male and female groups exposed to 30 mg/kg-day APFO. For this effect, the most appropriate dose metric is the estimated AUC for the 4-week old rat exposed to 30 mg/kg-day APFO; these estimates are 2,022 and 1,383 μg-hr/mL for the males and females, respectively.

The most appropriate human population for comparison is the children of ages 2-12 (Table 28). The AUC for human children calculated from the geometric mean is 0.1176 μg-hr/mL (0.0049 μg/mL X 24 hours), and the AUC for the 90th percentile is 0.204 μg-hr/mL (0.0085 μg/mL X 24 hours). MOEs were calculated using the gender specific estimates for the AUCs for the 4-week rat weanlings and the AUC for children. The MOEs calculated from the AUCs for male and female rat weanlings, respectively, and the AUC for the geometric mean for children are 17,194 and 11,760, respectively. If the AUC for the 90th percentile for the human children is used, the resulting MOEs are 9,912 and 6,779, respectively.

Another effect that was observed in the two-generation reproductive toxicity study was a delay in the sexual maturation of the F1 females exposed to 30 mg/kg-day APFO. Mean body weights were also reduced in this group prior to sexual maturation. Since sexual maturation occurs at approximately 5 weeks of age, the most appropriate estimate of serum concentrations is the average of the estimated AUCs for the 4 and 5 week female weanling exposed to 30 mg/kg-day (Appendix A) which is 1233 μg-hr/mL. The MOE calculated using the average AUC for the 4 and 5 week female weanling rat and the AUC for the geometric mean for human children is 10,485 (6,044 using the AUC for the 90th percentile).

Sexual maturation was also delayed in the F1 males in the two-generation reproductive toxicity study exposed to 30 mg/kg-day APFO. In addition, mean body weights were reduced prior to

sexual maturation in F1 males exposed to 10 and 30 mg/kg-day. Sexual maturation occurs at approximately 7 - 7.5 weeks, and the most appropriate estimate of serum concentrations is the average of the estimated AUCs for the 4 - 8 week male weanling (Appendix A). The average AUC at 10 mg/kg-day is 9237 μg-hr/mL. The MOE calculated from the average AUC for the 4 - 8 week male weanling rat and the AUC for human children is 78,546 (45,279 using the AUC for the 90th percentile).

5.6 Uncertainties in the Risk Characterization

This assessment relies on a rich hazard data base for an industrial chemical. Extensive pharmacokinetic information is available for rats through several life stages, and more limited information is available for humans and non-human primates. Similarly, there are animal toxicology studies for a variety of species including non-human primates, rats, mice and rabbits, and more limited epidemiological studies. Extensive mode of action information is available that has established that the liver toxicity and liver tumors are due to PPARα-agonism. In addition, serum biomonitoring information is available for humans throughout most of the life stages. PFOA serum levels were also obtained in some of the animal toxicology studies, and pharmacokinetic data permitted the estimation of serum levels in some cases where measured values were not available. These data permitted an analysis of potential human health effects associated with exposure to PFOA utilizing a variety of animal models, several life stages, and fairly precise estimates of actual human exposure.

Nonetheless, there are several uncertainties in this assessment. These are discussed below in a qualitative fashion, since it is premature to attempt a quantitative analysis at this time. One area of uncertainty pertains to the potential carcinogenesis associated with APFO exposure. In rats, chronic exposure to APFO results in liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumors. An extensive mode of action analysis has demonstrated that the liver tumors result from PPARα-agonism. However, there are insufficient information to establish the mode of action for the Leydig cell and pancreatic acinar cell tumors. In addition, there is some uncertainty regarding the development of mammary fibroadenomas following chronic APFO exposure in rats.

In addition, it is not known which animal species is the most appropriate model for humans. The prenatal developmental toxicity study in New Zealand White rabbits and the studies of immunotoxicity in mice were not considered in the risk assessment since serum levels of PFOA were not measured and there were no pharmacokinetic studies available with which to estimate serum levels. In addition, the female rat may not be a good model for humans due to the gender specific differences in elimination in rats. Information that is available for humans indicates that there is no gender difference in elimination. Thus, human females would be expected to be presented with a fairly continuous internal exposure to PFOA due to the long half-life, whereas the female rat experiences an internal exposure that is rapid and discontinuous because of the short half-life. This may be of particular importance in delineating the potential hazards associated with exposure to PFOA during development. Indeed, the mouse does not appear to have a gender difference in the elimination of PFOA, and preliminary results of a developmental toxicity study in CD-1 mice indicate early pregnancy loss, compromised postnatal survival and delays in postnatal growth and maturation following prenatal exposure to PFOA (Lau et al., 2004).

Non-human primate data were used to assess the potential health effects to adult humans. There is some uncertainty associated with the two studies that have been conducted due to the small sample size and the lack of a NOAEL. There is also some uncertainty associated with the exact

effects in the cynomolgus monkey study as it is not clear whether treatment-related mortality occurred in the lower dose groups. Finally, there is some uncertainty regarding the serum levels of PFOA in the cynomolgus monkey study due to the lack of a linear increase in serum PFOA levels in proportion to dose, the high variability, and the precision of the method itself which was ± 30% (inter-assay, intra-assay, and system).

Data from several toxicology studies in adult rats were also used to estimate the potential for health effects in adult humans. The only consistent effect that was noted in adult animals was a reduction in body weight. The implications of this effect for human health are not clear, but the endpoint was used to provide a conservative benchmark from a second species. As noted above, the female rat may not be a good model for human females. It is possible, due to the kinetics in the female rat, that some effects were not observed in the rat studies that may be observed in species with a longer half-life. In addition, there was a dose-related increase in the incidence of ovarian tubular hyperplasia in the 2-year study in rats. This effect was not observed in studies of shorter duration and did not progress to tumor formation in the 2-year study. Thus, the significance of this finding is not clear. Further research will need to be conducted to determine the significance of the finding for humans.

Several developmental effects associated with PFOA exposure were observed in the two-generation reproductive toxicity study in rats. The study did not determine the critical period of exposure for the developmental effects and serum levels were not measured. Therefore, it was necessary to use pharmacokinetic data to estimate the serum levels for the prenatal, lactation, and post-weaning periods. For the prenatal period, dose metrics were calculated for the pregnant dams; dose metrics were not calculated for the embryo/fetus since comparable dose metrics do not exist for humans. As described in Appendix A, pharmacokinetic data in adult female rats were used to estimate values for the volume of distribution, and elimination and absorption rate constants; this information was then used to estimate the AUC in the pregnant rat. Although one study did not report a difference in the elimination of PFOA during pregnancy in the rat (Gibson and Johnson, 1983), there is still some uncertainty in whether the kinetics are the same in the pregnant and non-pregnant rat. There is also some uncertainty whether the distribution of PFOA to the embryo/fetus is similar in rats and humans.

Unfortunately, dose metrics during the lactation period were not calculated. Therefore, it is not known what the potential MOE may be for this exposure period. Methods to estimate the dose to the offspring during lactation are under development.

Dose metrics for the post-weaning period were calculated. As described in Appendix A, data for post-weaning rats permitted estimation of the elimination constants during the post-weaning period. The adult volume of distribution and absorption constants were used; thus, there is some uncertainty whether the volume of distribution and absorption constants apply across all life stages.

The pharmacokinetic modeling described in Appendix A considered both one and two compartment models. Given the substantial database created for single dose pharmacokinetic studies in the rat, there would be expected to be relatively small uncertainties estimating AUCs. In general the fits to the plasma time course data were not substantially improved with the two compartment model as indicated by generally similar AIC values (see Tables A-xii and A-xv). Given the increased number of parameters in the two compartment model AIC values would have to be substantially lower for that model to give a statistically improved fit. Visual inspection showed that the divergence in plasma time course from the one compartment fit tended to occur at later times in some dose groups. With the females, it was impossible to obtain two-

compartment fits at lower concentrations due to rapid clearance and analytical detection limits. Given the relatively short period of dosing for the reproductive/developmental studies, error introduced through use of the one compartment model would be relatively small. For the male animals, doses that appeared well fitted by one compartment were interspersed with doses suggestive of a second compartment. Use of the one compartment model to estimate AUCs for chronic duration studies may tend to introduce greater error, though the greater uncertainty for the chronic studies is whether extended dosing alters physiology and thus pharmacokinetics.

There is also some uncertainty regarding the use of the human biomonitoring data. Although the available data include a range of populations with various demographic features, there may be some populations that are not represented. Since it is unknown how the human exposures are occurring, proximity to a manufacturing plant may be a factor in exposure. However, populations living near the plants were not sampled. Therefore, it is possible that PFOA serum levels may be underestimated for certain portions of the U.S. population. The children's sample was derived from blood collected in 1994/1995; therefore, it may not reflect the current status of PFOA in children's blood. It is not clear how PFOA may affect more sensitive subpopulations or if their exposures would vary. PFOA will be included in future environmental reports of the National Health and Nutrition Examination Survey (NHANES), in an effort to acquire nationally representative serum PFOA levels; however, children's serum levels will not be included.

Finally, there is some uncertainty in the interpretation of the MOEs. MOEs are generally calculated by dividing an administered dose in an animal study by an estimate of external human exposure. The value of the MOE that is associated with a concern for toxic effects is generally expressed as the product of the applicable uncertainty factors. The uncertainty factors that are considered are the same as those considered in the derivation of a RfD or RfC. Generally this includes consideration of an uncertainty factor for intraspecies variation and another for interspecies variation. An additional factor may be considered if a LOAEL is used from the animal toxicology study. In this assessment, MOEs were calculated from serum levels in animals and humans. Generally, data are not available for this kind of estimate, and therefore there is little experience in determining whether the same uncertainty factors apply. In addition, this assessment is unique in that the half-life of PFOA is very different among the various animal species and humans. Whether this should be a consideration in interpreting the MOE is not clear.

6.0 Overall Conclusions

This risk assessment focused on the potential human health effects associated with exposure to PFOA and its salts. The results of existing epidemiology studies are not adequate for use in quantitative risk assessment, and therefore the analysis was restricted to endpoints in the animal toxicology studies. MOEs were calculated for the general U.S. population. Although some serum level data were available for workers, the data were not adequate to calculate MOEs for specific occupational exposures. In general, the mean serum levels following occupational exposures appear to be orders of magnitude higher than observed in the general population. Thus, MOEs for workers would be expected to be much less than for the general population.

A variety of endpoints from the animal toxicology studies were used to calculate MOEs for this draft risk assessment. The endpoints encompassed different species, gender and life stages. For this draft assessment, specific recommendations on the most appropriate endpoint/lifestage/species/gender have not been made; rather, all have been presented to provide transparency.

For adults, two sets of MOEs were calculated based on the toxicology studies in non-human primates and rats. First, calculation of MOEs from the cynomolgus monkey study was based on increased liver weight and possible mortality. The MOE using the geometric mean for the human serum level is 16,739 (8,191 for the 90th percentile). Second, calculation of MOEs from the adult rat studies was based on reductions in body weight. MOEs were calculated separately for the female and male rat due to the gender differences in pharmacokinetics in this species. MOEs were calculated by dividing the AUC in the adult female rat by the AUC for the adult humans which is 398 (195 for the 90th percentile) and by dividing the AUC for the adult male rat by the AUC for the adult humans which is 9158 (4481 for the 90th percentile).

MOEs were calculated for the developmental effects in the two-generation reproductive toxicity study in rats. These effects were observed at various times during the maturation of the F1 pups; the critical period of exposure for each of the effects is not known, and could be due to exposure during the prenatal, lactational, and/or postweaning periods. Ideally, MOEs should be calculated for each of these exposure periods; however MOEs were not calculated for the lactation period due to uncertainties in pharmacokinetics. For the prenatal period, MOEs were calculated for the pregnant human female; MOEs were not calculated for the fetus since there is no information on human serum levels in fetuses. MOEs were calculated using both C_{max} and AUC; the MOE based on C_{max} is 3,095 (1548 for the 90th percentile) and the MOE based on the AUC is 823 (412 for the 90th percentile).

For the postweaning period, MOEs were calculated for several endpoints including reductions in body weight, mortality and delayed sexual maturation. These MOEs were based on the geometric mean for children and range from 10,484 - 78,546 (the range using the 90th percentile is 6,044 - 45,279).

This assessment has provided a range of MOEs for several life stages. Several uncertainties have been discussed in a qualitative fashion in this assessment, which highlight the need to interpret the MOEs with caution. For example, MOEs were not calculated for the lactation period due to insufficient data, and this life stage may represent an important exposure period. Similarly, the biomonitoring data for the children are from samples collected in 1994 and may not be representative of current children's serum levels. In addition, ascertaining potential levels of concern will necessitate a better understanding of the appropriate dose metric in rats or other animal models, and the relationship of the dose metric to the human serum levels.

Finally, there is some uncertainty associated with the determination of the adequacy of a specific MOE in protecting human health in the present context. Generally, MOEs are calculated from administered dose levels and estimates of human exposure. In this assessment, the MOEs were calculated from internal dose metrics in animals and humans. While use of internal dose metrics reduces many uncertainties pertaining to exposure, there is little experience or guidance on the factors that should be considered in making judgements about the level of concern associated with a given MOE. Approaches that are used for conventional MOEs, if applied unchanged, indicate that among the populations of interest some individuals are highly exposed, for reasons not understood at this time. However, if conventional approaches for determining levels of concern are not appropriate for MOEs based on internal dose metrics, then this conclusion would have to be re-evaluated as the understanding of this question evolves.

7.0 References

3M (Minnesota Mining and Manufacturing Company). 1999a Letter (dated May 26, 1999) re: TSCA 8(e) Supplemental Notice: Sulfonate-based and carboxylic-based fluorochemicals–Docket Nos. 8EHQ-1180-373; 8EHQ-1180-374; 8EHQ-0381-0394; 8EHQ-0699-373.

3M (Minnesota Mining and Manufacturing Company). 1999b The Science of Organic Fluorochemistry" and "Perfluorooctane sulfonate: current summary of human sera, health, and toxicology data." February 5, 1999. 8EHQ-0299-373.

3M (Minnesota Mining and Manufacturing Company). 2001 Material Safety Data Sheet FC-26 FLUORAD Brand Fluorochemical Acid, ID Number/U.P.C.: ZF-0002-0376-8. Online: http://www.3m.com/US/safety/index.jhtml.

Abdellatif, A.G.; Preat, V.; Taper, H.S. and Roberfroid, M. 1991 The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. Toxicol. Appl. Pharmacol. 111:530-537.

Alexander, B.H. 2001a Mortality study of workers employed at the 3M Cottage Grove facility. Final Report. April 26, 2001. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota. US EPA AR226-1030a018.

Alexander, B.H. 2001b Mortality study of workers employed at the 3M Decatur facility. Final Report. April 26, 2001. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota. US EPA AR226-1030a019.

Berthiaume, J. and Wallace, K.B. 2002 Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. Toxicol. Lett. 129:23-32.

Biegel, L.B.; Liu, R.C.M; Hurtt, M.E. and Cook, J.C. 1995 Effects of ammonium perfluorooctanate on Leydig cell function: in vitro, in vivo, and ex vivo studies. Toxicol. Appl. Pharmacol. 134:18-25.

Biegel, L.B.; Hurtt, M.E.; Frame, S.R.; O'Conner, J.C. and Cook, J.C. 2001 Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol. Sci. 60:44-55.

Bluist, S.; Cherrington, N.J; Choudhuri, J.; Hartley, D.P. and Klaassen, C.D. 2002 Gender-specific and developmental influences on the expression of rat organic anion transporters. J. Pharmacol. Exper. Ther. 301:145-151.

Boit, H-G., Editor. 1975 Beilstein Handbook of Organic Chemistry. Springer-Verlag, Berlin. 4th Work, Volume 2, Part 2, page 994.

Burris, J.M.; Olsen, G.; Simpson, C. and Mandel, J. 2000 Determination of serum half-lives of several fluorochemicals. 3M Company. Interim Report #1. June 8, 2000. US EPA AR226-0611.

Burris, J.M.; Lundberg, J.K.; Olsen, G.W.; Simpson, D. and Mandel, G. 2002 Determination of serum half-lives of several fluorochemicals. 3M Company. Interim Report #2. January 11, 2002. US EPA AR226-1086.

Butenhoff, J.; Costa, G.; Elcombe, C.; Farrar, D.; Hansen, K.; Iwai, H.; Jung, R.; Kennedy, G.; Lieder, P.; Olsen, G. and Thomford, P. 2002 Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicol. Sci. 69: 244-257.

Butenhoff, J.L.; Kennedy, G.L.; Frame, S.R.; O'Conner, J.C. and York, R.G. 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology. 196:95-116.

Calfours, J. and Stilbs, P. 1985 Solubilization in sodium perfluorooctanoate micelles: a multicomponent self-diffusion study. Colloid Interface Sci. 103:332-336.

Cattley, R.C.; DeLuca, J.; Elcombe, C.; Fenner-Crisp, P.; Lake, B.G.; Marsman, D.S.; Pastoor, T.A.; Popp, J.A.; Robinson, D.E.; Schwetz, B.; Tugwood, J. and Wahli, W. 1998 Do peroxisome proliferating compounds pose a hepatocarcinogenic risk to humans? Regul. Toxicol. Pharmacol. 27:47-60.

Cameron, R.G.; Imaida, K.; Tsuda, H.; and Ito, N. 1982 Promotive effects of steroids and bile acids on hepatocarcinogenesis initiated by diethylnitrosamine. Cancer Res. 42:2426-2428.

Chandra, M.; Riley, M.G.I.; and Johnson, DE. 1992 Spontaneous neoplasms in aged sprague-dawley rats. Arch. Toxiocol. 66:496-502.

Chapin, R.E.; Stevens, J.T.; Hughes, C.L.; Kelce, W.R.; Hess, R.A.;and Daston, G.P. 1996 Symposium overview: endocrine modulation of reproduction. Fund. Appl. Toxicol. 29:1-17.

Cheung, C., Akiyama, TE, Ward, JM, Nicol, CJ, Feigenbaum, L., Vinson, C., and Gonzalez, FJ. 2004. Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor α. Cancer Res. 64:3849-3854.

Christopher, B. and Marias, A.J. 1977 28-Day oral toxicity study with FC-143 in albino mice, Final Report, Industrial Bio-Test Laboratories, Inc. Study No. 8532-10655, 3M Reference No. T-1742CoC, Lot 269.

Clegg, E.D.; Cook, J.C.; Chapin, R.E.; Foster, P.M.D.; and Daston, G.P. 1997 Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reproduct. Toxicol. 11:107-121.

Cook, J.C.; Hurtt, M.E.; Frame, S.R. and Biegel, L.B. 1994 Mechanisms of extrahepatic tumor induction by peroxisome proliferators in Crl:CD BR(CD) rats. Toxicologist, 14:301, (abstract # 1169).

Cook, J.C.; Klinefelter, G.R.; Hardisty, J.F.; Sharpe, R.M. and Foster, P.M. 1999. Rodent Leydig cell tumorigenesis: A review of physiology, pathology, mechanisms and relevance to humans. Crit. Rev. Toxicol. 29:169-261.

Cotran, R.S.; Kumar, V. and Robbins, S.L. 1989 The pancreas. In: Pathologic Basis of Disease, pp. 981-1010. W.B. Saunders, Philadelphia.

Cutts, J.H. and Noble, R.L. 1964 Estrone-induced mammary tumors in the rat. I. Induction and behavior of tumors. Cancer Res. 24:1116-1123.

Daikin Chemicals Sales Co., LTD. 2001 Online Catalog. Fluoro aliptiatic compounds. http://daikin-dcs.co.jp/catalog/

Dean, W.P. and Jessup, D.C. 1978 Acute oral toxicity (LD50) study in rats. International Research and Development Corporation, Study No. 137-091, May 5, 1978. US EPA AR226-0419.

Dupont (Haskell Laboratory). 2001a Letter to Charles Auer from Gerald Kennedy, dated January 25, 2001. FYI-00-001378. US EPA AR226-1000.

Dupont (Haskell Laboratory). 2001b Letter from Gerald Kennedy re: FYI-010-01378 and FYI-0301-01378, dated May 29, 2001. US EPA AR226-1023.

Dupont (Haskell Laboratory). 2003 Epidemiology surveillance report: Cancer incidence for Washington works site 1959-2001. US EPA AR226-1307-6.

Eagon, P.K;, Chander, N.; Epley, M.J.; Elm, M.S.; Brady, E.P. and Rao, K.N. 1994. Di(2-ethylhexyl)phthalate-induced changes in liver estyrogen metabolism and hyperplasia. Int. J. Cancer 58:736-743

Edwards, P.J.B; Jolley, K.W.; Smith, M.H.; Thomsen, S.J.; and Boden, N. 1997 Solvent isotope effect on the self-assembly liquid crystalline phase behavior in aqueous solutions of ammonium pentadecafluorooctanoate.. Langmuir:13(10):2665-2669.

Exfluor Research Company. 1998 Material Safety Data Sheet for Pentadecafluorooctanoic acid, 96%, Acros Organics N.V. Online: http://216.122.170.58/msds.asp?ExfID=C8AC

FIFRA SAP. 2004 Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel meeting held December 9, 2003. Memo. from S. M. Knott to J.J. Jones and C.M. Auer, March 5, 2004. Office of Science Coordination and Policy, U.S. Environmental Protection Agency, Washington, DC.

Fisher Scientific. 2003 Material Safety Data Sheet for C8AC, Perfluorooctanoic acid, 98%. Online: https://fscimage.fishersci.com/msds/97331.htm

Frame, S.R. and McConnell, E.E. 2003 Review of proliferative lesions of the exocrine pancreas in two chronic studies in rats with ammonium perfluorooctanoate. DuPont-13788. Oct. 16, 2003.

Gabriel, K. 1976a Primary eye irritation study in rabbits. Biosearch, Inc., March 4, 1976. US EPA AR226-0422.

Gabriel, K. 1976b Primary skin irritation study in rabbits. Biosearch, Inc., March 4, 1976. US EPA AR226-0423.

Gabriel, K. 1976c Acute oral toxicity - rats. Biosearch, Inc., September 16, 1976. US EPA AR226-0425.

Gabriel, K. 1976d Primary eye irritation study in rabbits. Biosearch, Inc., September 16, 1976. US EPA AR226-0426.

Garry, V.F. and R.L. Nelson. 1981 An assay of cell transformation and cytotoxicity in C3H10T½ clonal cell line for the test chemical T-2942 CoC. Stone Research Laboratories, Minneapolis, MN, March 4, 1981. US EPA AR226-0428.

Gazouli, M.; Yao, Z.X.; Boujard, N.; Corton, J.C.; Culty, M. and Papadopoulos, V. 2002.Effect of peroxisome proliferators on Leydig cell periferal-type benzodiazepine receptor gene expression, hormone-stimulated chloresterol transport, and steroidogenesis: Role ofthe proxisome proliferator-activated receptor α. Endocrinology 143:2571-2583.

Gibson, S.J. and Johnson, J.D. 1979 Absorption of FC-143-14C in rats after a single oral dose. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, Minnesota.

Gibson, S.J. and Johnson, J.D. 1980 Extent and route of excretion and tissue distribution of total carbon-14 in male and female rats after a single I.V. dose of FC-143-14C. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN.

Gibson, S.J. and Johnson, J.D. 1983 Extent and route of excretion of total carbon-14 in pregnant rats after a single oral dose of ammonium 14 C-perfluorooctanoate. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN.

Glaza, S. 1995 Acute dermal toxicity study of T-6342 in rabbits. Corning Hazelton, Inc. Madison, WI. Project ID: HWI 50800374. 3M Company. St. Paul, MN. US EPA AR226-0427.

Glaza, S.M. 1997 Acute oral toxicity study of T-6669 in rats. Corning Hazleton Inc. Study No. CHW 61001760, January 10, 1997. US EPA AR226-0420.

Gilliland, F. 1992 Fluorochemicals and human health: Studies in an occupational cohort. Doctoral thesis, Division of Environmental and Occupational Health, University of Minnesota. US EPA AR226-0473.

Gilliland, F.D. and Mandel, J.S.. 1993 Mortality among employees of a perfluorooctanoic acid production plant. J. Occup. Med. 35(9):950-954.

Gilliland, F.D. and Mandel, J.S. 1996 Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: A study of occupationally exposed men. Am. J. Ind. Med. 29:560-568.

Goldenthal, E.I. 1978a Final Report, Ninety Day Subacute Rat Toxicity Study on Fluorad® Fluorochemical FC-143, International Research and Development Corporation, Study No. 137-089, 3M Reference No. T-3141, November 6, 1978. US EPA AR226-0441.

Goldenthal, E.I. 1978b Final Report, Ninety Day Subacute Rhesus Monkey Toxicity Study, International Research and Development Corporation, Study No. 137-090, November 10, 1978. US EPA AR226-0447.

Gortner, E.G. 1981 Oral teratology study of T-2998CoC in rats. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0681TR0110, December 1981.

Gortner, E.G. 1982 Oral teratology study of T-3141CoC in rabbits. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0681TB0398, February 1982.

Griffith, F.D. and Long, J.E. 1980 Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J 41(8):576-583.

Han, H.; Snow, T.A.; Kemper, R.A. and Jepson, G.W. 2003 Binding of perfluorooctanoic acid to rat and human plasma proteins. Chem. Res. Toxicol. 16:775-781.

Han, X. 2003 Ammonium perfluorooctanoate: Age effect on the plasma concentration in post-wealning rats following oral gavage. Haskell Laboratory for Health and Environmental Sciences. Study No. Dupont-13267, December 15, 2003 US EPA AR226-1553

Hanhijarvi, H.; Ophaug, R.H. and Singer, L. 1982 The sex-related difference in perfluorooctanoate excretion in the rat. Proc. Soc. Exp. Biol. Med. 171:50-55.

Hanhijarvi, H.; Ylinen, M.; Kojo, A. and Kosma, V.M. 1987 Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. Pharmacol. Toxicol. 61:66-68.

Hanhijarvi, H.; Ylinen, M.; Haaranen, T. and Nevalainen, T. 1988 A proposed species difference in the renal excretion of perfluorooctanoic acid in the beagle dog and rat. In: New Developments in Biosciences: Their Implications for Laboratory Animal Science. Beynen, AC; Solleveld, HA, Eds. Martinus Nijhoff Publishers. Dordrecht, Netherlands.

Hansch, C. and Leo, A.; Eds. 1979 The fragment method of calculated partition coefficients. In: Substituent Constants for Correlation Analysis and Chemistry and Biology, Chapter I.V. John Wiley and Sons, Inc., New York.

Hinderliter, P.M. 2003 Perfluorooctanoic acid: relationship between repeated inhalation exposures and plasma PFOA concentration in the rat. Haskell Laboratory for Health and Environmental Sciences. Study No. DuPont-12944, November 5, 2003.

IARC (International Agency for Research on Cancer). 1995 WHO International Agency for Research on Cancer. Peroxisome proliferation and its role in carcinogenesis. IARC technical report No. 24. IARC Press, Lyon, France.

Ikeda, T.; Aiba, K.; Fukuda, K. and Tanaka, M. 1985 The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. J. Biochem. 98:475-482.

Johnson, J.D. 1995a Final report, analytical study, single-dose intravenous pharmacokinetic study of T-6067 in rabbits. Study Number: AMDT-120694.1. 3M Environmental Technology & Services, St. Paul, MN. US EPA AR226-0453.

Johnson, J.D. 1995b Final report, analytical study, single-dose absorption/toxicity study of T-6067, T-6068, and T-6069 in rabbits. Study Number: AMDT-011095.1. 3M Environmental Technology & Services, St. Paul, MN. US EPA AR226-0454.

Johnson, J.D.; Gibson, S.J. and Ober, R.E. 1984 Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C]perfluorooctanoate or potassium [^{14}C]perfluorooctanesulfonate. Fundam. Appl. Toxicol. 4:972-976.

Karns, M.E. and Fayerweather, W.E. 1991 A case-control study of leukemia at the Washington Works site. Final Report. Dupont Company. December 31, 1991. US EPA AR226-1308-2.

Kawashima, Y., Uy-Yu, N. and Kozuka, H. 1989 Sex-related difference in the inductions by perfluoro-octaanoic acid of peroxisome â-oxidation, microsomal 1-acylglycerolphosphocholine acyltransferase and cytosolic long-chain acyl-CoA hydrolase in rat liver. Biochem. J. 261:595-600.

Keller, B.J.; Marsman, D.S.; Popp, J.A. and Thurman, R.G. 1992 Several nongenotoxic carcinogens uncouple mitochondrial oxidative phosphorylation. Biochem. Biophys. Acta, 1102:237-244.

Kemper, R.A. 2003 Perfluorooctanoic acid: Toxicokinetics in the rat. UE EPA AR 116.

Kennedy, G.L. 1985 Dermal toxicity of ammonium perfluorooctanoate. Toxicol. Appl. Pharmacol. 81(2):348-355.

Kennedy, G.L.; Hall, G.T.; Brittelli, M.R.; Barnes, J.R. and Chen, H.C. 1986 Inhalation toxicity of ammonium perfluorooctanoate. Food Chem. Toxicol. 24(12):1325-1329.

Klaunig, J.E.; Babich, M.A.; Baetcke, K.P.; Cook, J.C.; Corton, J.C.; David, R.M.; DeLuca, J.G.; Lai, D.Y.; McKee, R.H.; Peters, J.M.; Roberts, R.A. and Fenner-Crisp, P.A. 2003 PPARα agonist-induced rodent tumors: Mode of action and human relevance. Crit. Rev. Toxicol. 33:655-780.

Kudo, N.; Katakura, M.; Sato, Y. and Kawashima, Y. 2002 Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem. Biol. Interact. 139:301-316.

Lau,C.; Thibodeaux, J.R.; Hanson, R.G. and Rogers. J.M. 2004 Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Birth Defects Research, IN PRESS

Lawlor, T.E. 1995 Mutagenicity test with T-6342 in the *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay. Laboratory Number: 17073-0-409. Corning Hazleton Inc., Vienna, VA. 3M Company. St. Paul, MN. US EPA AR226-0436.

Lawlor, T.E. 1996 Mutagenicity test with T-6564 in the *Salmonella–Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay. Corning Hazleton Inc. Final Report. CHV Study No: 17750-0-409R. September 13, 1996. US EPA AR226-0432.

Lines, D. and Sutcliffe, H. 1984 Preparation and properties of some salts of perfluorooctanoic acid. Journal of Fluorine Chemistry 25:505-512.

Liu, R.C.M.;, Hurtt, M.E.; Cook, J.C. and Biegel, L.B. 1996 Effect of the peroxisome proliferator, ammonium perfluorooctanate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. Fund. Appl. Toxicol. 30:220-228.

Liu, S.C.; Sanfilippo, B.; Perroteau, I.; Derynck, R.; Salomon, D.S. and Kidwell, W.R. 1987 Expression of transforming growth factor alpha (TGF alpha) in differentiated rat mammary tumors: estrogen induction of TGF alpha production. Mol. Endocrinol. 1:683-692

Longnecker, D.S. 1987 Interface between adaptive and neoplastic growth in the pancreas. Gut 28:253-258

Longnecker, D.S. and Sumi, C. 1990 Effects of sex steroid hormones on pancreatic cancer in the rat. Int. J. Pancreatol. 7:159-165.

Luebker, D.J.; Hansen, K. J.; Bass, N.M.; Butenhoff, J.L. and Seacat, A.M. 2002 Interactions of fluorochemicals with rat liver fatty acid-binding proteins, Toxicology: 176:175-185.

Maloney, E.K. and Waxman, D.J. 1999 Transactivation of PPARα and PPARγ by structurally diverse environmental chemicals. Toxicol. Appl. Pharmacol. 161:209-218.

Mann, P.C. and Frame, S.R. 2004 FC-143: Two year oral toxicity-oncogenicity study in rats: Peer review of ovaries. DuPont Project ID 15261, June 25, 2004. US EPA AR226.

Markoe, D.M. 1983 Primary skin irritation test with T-3371 in albino rabbits. Riker Laboratories, Study No.0883EB0079, July 13, 1983. US EPA AR226-0424.

Merck KgaA. Undated. Chemical/physical Data, 807114, Pentadecafluorooctanoic acid for synthesis. Online: http://www.chemdat.de/cat/web2.zoom_in?text=807114&screen=120&cid=-788291649&pg=0&s=pentadecafluorooctanoic&lang=4

Metrick, M. and Marias, A.J. 1977 28-Day Oral Toxicity Study with FC-143 in Albino Rats, Final Report, Industrial Bio-Test Laboratories, Inc. Study No. 8532-10654, 3M Reference No. T-1742CoC, Lot 269, September 29, 1977

Murli, H. 1995 Mutagenicity test on T-6342 in an in vivo mouse micronucleus assay. Corning Hazleton Inc., Vienna, VA. Study No. 17073-0-455, December 14, 1995. US EPA AR226-0435.

Mylchreest E. 2003 PFOA: Lactational and placental transport pharmacokinetic study in rats. Haskell Laboratory for Health and Environmental Sciences, Newark DE, Study No. DuPont-13309, December 19, 2003. US EPA AR226-1551

Murli, H. 1996a Mutagenicity test on T-6564 in an in vivo mouse micronucleus assay. Corning Hazleton Inc., Vienna, VA. Study number 17750-0-455, November 1, 1996. US EPA AR226-0430.

Murli, H. 1996b Mutagenicity test on T-6564 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. Corning Hazleton Inc., Vienna, VA. Study No. 17750-0-437CO, September 16, 1996. US EPA AR226-0431.

Murli, H. 1996c Mutagenicity test on T-6342 measuring chromosomal aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. Corning-Hazelton, Inc., Vienna, VA. Study No. 17073-0-449CO, November 1, 1996. US EPA AR226-0433.

Murli, H. 1996d Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. Corning-Hazelton, Inc. Vienna, VA. Study No. 17073-0-437CO, September 16, 1996. US EPA AR226-0434.

Nilsson, R.; Beije, B.; Preat, V.; Erxon, K. and Ramel, C. 1991 On the mechanism of the hepatocarcinogenicity of peroxisome proliferators. Chem. Biol. Interact. 78:235-250.

NIOSH (National Institute of Occupational Safety and Health). 1994 International Chemical Safety Card, Chloroacetic Acid, ICSC: 2035, IPCS, CEC. Online: http://www.cdc.gov/niosh/ipcsneng/neng0235.html

NOTOX. 2000 Evaluation of the ability of T-7524 to induce chromosome aberrations in cultured peripheral human lymphocytes. NOTOX Project Number 292062. Hertogenbosch, The Netherlands.

Obourn, J.D.; Frame. S.R.; Bell, R.H. Jr.; Longnecker, D.S.; Elliott, G.S. and Cook, J.C. 1997 Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14,643. Toxicol. Appl. Pharmacol. 145:425-436.

O'Flaherty, E.J. 1981 Toxicants and Drugs: Kinetics and Dynamics. John Wiley and Sons, New York.

Olsen, G.W.; Gilliland, F.D.; Burlew, M.M.; Burris, J.M.; Mandel, J.S. and Mandel J.H. 1998a An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. JOEM 40(7):614-622.

Olsen, G.W.; Burris, J.M.; Burlew, M.M.; Mandel, J.H. 1998b 3M Final report: an epidemiologic investigation of plasma cholecystokinin, hepatic function and serum perfluorooctanoic acid levels in production workers. 3M Company. September 4, 1998. US EPA AR226-0476.

Olsen, G.W.; Burris, J.M.; Mandel, J.H.; Zobel, L.R. 1998c An epidemiologic investigation of clinical chemistries, hematology and hormones in relation to serum levels of perfluorooctane sulfonate in male fluorochemical production employees. 3M Company. Final Report. August 22, 1998. US EPA AR226-0030.

Olsen, G.W.; Logan, P.W.; Simpson, C.A.; Hansen, K.J.; Burris, J.M.; Burlew, M.M.; Schumpert, J.C. and Mandel, J.H.. 1999 Fluorochemical exposure assessment of Decatur chemical and film plant employees. 3M Company. Final Report. August 11, 1999. US EPA AR226-0950.

Olsen, G.W.; Burris, J.M.: Burlew, M.M. and Mandel, J.H. 2000 Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drug Chem Tox 23(4):603-620.

Olsen, G.W.; Logan, P.W.; Simpson, C.A.; Burris, J.M.; Burlew, M.M.; Lundberg, J.K.; and Mandel, J.H. 2001a Descriptive summary of serum fluorochemical levels among employee participants of the year 2000 Decatur fluorochemical medical surveillance program. 3M Company. Final Report. March 19, 2001. US EPA AR226-1030a020a.

Olsen, G.W.; Schmickler, M.N.; Tierens J.M.; Logan P.W.; Burris, J.M.; Burlew, M.M.; Lundberg, J.K.; Mandel, J.H. 2001b Descriptive summary of serum fluorochemical levels among employee participants of the year 2000 Antwerp fluorochemical medical surveillance program. 3M Company. Final Report. US EPA AR 226-1030a020b.

Olsen, G.W.; Madsen, D.C.; Burris, J.M. and Mandel, J.H. 2001c Descriptive summary of serum fluorochemical levels among 236 building employees. 3M Company. Final Report. March 19, 2001. US EPA AR226-1030a020c.

Olsen, G.W.; Hansen, K.J.; Clemen, L.A.; Burris, J.M. and Mandel, J.H. 2001d Identification of Fluorochemicals in Human Tissue. 3M Company. Final Report. June 25, 2001. US EPA AR226-1030a022.

Olsen G.W.; Burlew, M.M.; Burris, J.M. and Mandel, J.H. 2001e A cross-sectional analysis of serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology and urinalysis results from male and female employee participants of the 2000 Antwerp and Decatur fluorochemical medical surveillance program. 3M Company. Final Report. October 11, 2001. US EPA AR226-1087.

Olsen, G.W.; Burlew, M.M.; Burris, J.M..and Mandel, J.H. 2001f A longitudinal analysis of serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) levels in relation to lipid and hepatic clinical chemistry test results from male employee participants of the 1994/95, 1997, and 2000 fluorochemical medical surveillance program. 3M Company. Final Report. October 11, 2001. US EPA AR226-1088.

Olsen, G.W.; Burlew, M.M.; Hocking, B.B.; Skratt, J.C.; Burris, J.M. and Mandel, J.H. 2001g An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993-1998. 3M Company. Final Report. May 18, 2001. US EPA AR226-1030a021.

Olsen, G.W.; Burris, J.M.; Lundberg, J.K.; Hansen, K..; Mandel, J.H. and Zobel, L.R. 2002a Identification of fluorochemicals in human sera. I. American Red Cross adult blood donors. 3M Company. Final Report. February 25, 2002. US EPA AR226-1083.

Olsen, G.W.; Burris, J.M.; Lundberg, J.K.; Hansen, K.J.; Mandel, J.H. and Zobel, L.R. 2002b Identification of fluorochemicals in human sera. II. Elderly participants of the Adult Changes in Thought Study, Seattle, Washington. 3M Company. Final Report. February 25, 2002. US EPA AR226-1084.

Olsen, G.W.; Burris, J.M.; Lundberg, J.K.; Hansen, K.J.; Mandel, J.H. and Zobel, L.R. 2002c Identification of fluorochemicals in human sera. III. Pediatric participants in a Group A Streptococci clinical trial investigation. 3M Company. Final Report. February 25, 2002. US EPA AR226-1085.

Olsen, G.W. and Mandel, J.H. 2003a Descriptive analysis of serum fluorochemical concentrations from Cottage Grove employee participants of the 2002 Medical Surveillance Program. 3M Company. Final Report. US EPA AR226-1352.

Olsen, G.W. and Mandel, J.H. 2003b Descriptive analysis of serum fluorochemical concentrations from Decatur employee participants of the 2002 Medical Surveillance Program. 3M Company. Final Report. US EPA AR226-1353.

Olsen, G.W.; Church, T.R.; Miller, J.P.; Burris, J.M.; Hansen, K.J.; Lundberg J.K.; Armitage, J.B.; Herron, R.M.; Medhdizadehkashi, Z.; Nobiletti, J.B.; O'Neill, E.M.; Mandel, J.H.; and Zobel, L.R. 2003d Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. Environ Health Perspect 111(16):1892-1901.

Olsen, G.W.; Burris, J.M.; Burlew, M.M.; Mandel, J.H. 2003e Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. JOEM 45(3):260-270.

Olsen, G.W.; Butenhoff, J.L.; Mandel, J.H. 2003f Assessment of lipid, hepatic and thyroid function in relation to an occupational biologic limit value for perfluorooctanoate. 3M Company. Final Report. June 9, 2003. US EPA AR226-1351.

Olsen, G.W.; Church, T.R.; Larson E.B.; van Belle, G.; Lundberg J.K.; Hansen, K.J.; Burris J.M.; Mandel J.H, and Zobel, L.R. 2004a Serum concentrations of perfluorooctanesulfonate and other fluorochemicals in an elderly population from Seattle, Washington. Chemosphere 54:1599-1611.

Olsen, G.W.; Church, T.R.; Hansen, K.J.; Butenhoff, J.L.; Mandel, J.H.; and Zobel, L.R. 2004b Quantitative evaluation of perfluorooctanesulfonate (PFOS) and other FC's in the serum of children. J. Children's Health 2:1-24.

O'Malley, K.D. and Ebbins, K.L. 1981 Repeat application 28 day percutaneous absorption study with T-2618CoC in albino rabbits. Riker Laboratories, St. Paul, MN. US EPA AR226-0446.

Ophaug, R.H. and Singer, L. 1980 Metabolic handling of perfluorooctanoic acid in rats. Proc Soc Exp Biol Med 163:19-23.

OPPTS. 2003 Proposed science policy: PPARα-mediated hepatocarcinogenesis in rodents and relevance to human health risk assessments. Submitted to: FIFRA Scientific Advisory Panel (SAP). Office of Prevention, Pesticides & Toxic Substances, November 5, 2003. Available from <http://www.epa.gov/oscpmont/sap/2003/december9/peroxisomeproliferatorssciencepolicy.pdf>

Palazzolo, M.J. 1993 Thirteen-week dietary toxicity study with T-5180, ammonium perfluorooctanoate (CAS No. 3825-26-1) in male rats. Final Report. Laboratory Project Identification HWI 6329-100. Hazleton Wisconsin, Inc. US EPA AR226-0449.

Palmer, C.N.; Hsu, M.H;, Griffin, K.J;, Raucy, J.L. and Johnson, E.F. 1998 Peroxisome proliferator acitivated receptor-alpha expression in human liver. Mol. Pharmacol. 53:14-22.

Panaretakis, T.; Shabalina, I.G.; Grander, D.; Shoshan, M.C. and DePierre, J.W. 2001 Reactive oxygen species and mitochondria mediate the induction of apoptosis in human hepatoma hepG2 cells by the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid. Toxicol. Appl. Pharmacol. 173:56-64.

Pastoor, T.P.; Lee, K.P.; Perri, M.A. and Gillies, P.J. 1987 Biochemical and morphological studies of ammonium perfluorooctanate-induced hepatomegaly and peroxisome proliferation. Exp. Mol. Pathol. 47:98-109.

Prentice, D.E. and Meikle, A.W. 1995 A review of drug-induced Leydig cell hyperplasia and neoplasia in the rat and some comparisons with man. Human Exper. Toxicol. 14: 562-572.

Rusch, G. 1979 An acute inhalation study of T-2305 CoC in the rat. Bio/dynamics, Inc., Study No. 78-7184, May 3, 1979. US EPA AR226-0417.

Sadhu, D. 2002 CHO/HGPRT forward mutation assay – ISO (T6.889.7). Toxicon Corporation, Bedford, MA. Report No. 01-7019-G1, March 28, 2002. US EPA AR226-1101.

Sibinski, L.J. 1987 Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats. Vol.1-4, 3M Company/RIKER Exp. No.0281CR0012; 8EHQ-1087-0394, October 16, 1987.

Simister, E.A.; Lee, E.M.; Lu, J.R.; Thomas, R.K.; Ottewill, R.H.; Rennie, A.R. and Penfold, J. 1992 Adsorption of ammonium perfluoroocanoate and ammonium decanoate at the air-solution interface. J Chem. Soc., Faraday Trans. 88(20):3033-41.

Sohlenius, A-K.; Andersson, K. and DePierre, J.W. 1992 The effects of perfluoro-octanoic acid on hepatic peroxisome proliferation and related parameters show no sex-related differences in mice. Biochem. J. 285:779-783.

Sonich-Mullin, C.; Fielder, R.; Wiltse, J.; Baetcke, K.; Dempsey, J.; Fenner-Crisp, P.; Grant, D.; Hartley, M.; Knaap, A.; Kroese, D.; Mangelsdorf, I.; Meek, E.; Rice, J.M. and Younes, M. 2001 IPCS conceptual framework for evaluating a MOA for chemical carcinogenesis. Regul. Toxicol. Pharmacol. 34:146-152.

Southern Research Institute. 2003 Protein binding of perfluorohexane sulfonate, perfluorooctane sulfonate and perfluorooctanoate to plasma (human, rat and monkey), and various human derived plasma protein fractions. Study ID: 9921.7. US EPA AR226-1354.

Staples, R.E.; Burgess, B.A. and Kerns, W.D. 1984 The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundam Appl Toxicol 4:429-440.

Starkov, A.A. and Wallace, K.B. 2002 Structural determinants of fluorochemical-induced mitochondrial dysfunction. Toxicol. Sci. 66:244-252.

Sykes, G. 1987 Two-year toxicology/carcinogenicity study of fluorochemical FC-143 in rats. Memo. from G. Sykes to C. Reinhardt, Haskell Lab. Toxicol. Ind. Med., October 29, 1987.

Takagi, A; Sai, K.; Ummemura, T.; Hasegawa, R. and Kurokawa, Y. 1991 Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increases of 8-hydroxydeoxyguanosine in liver DNA of rats. Cancer Lett. 57:55-60.

Takagi, A.; Sai, K.; Ummemura, T.; Hasegawa, R.; and Kurokawa, Y. 1992 Hepatomegaly is an early biomarker for hepatocarcinogenesis induced by peroxisome proliferators. J. Environ. Toxicol. Pathol. 11:45-149.

Teerds, K.J.; Rommerts, F.G.; and Dorrington, J.H. 1990 Immunohistochemical detection of transforming growth factor-á in Leydig cell during the development of the rat testis. Molec. Cell Endocrinol. 69:R1-R6.

Thomford, P.J. 2001a 4-Week capsule toxicity study with ammonium perfluorooctanoate (APFO) in cynomolgus monkeys. Study performed by Covance Laboratories Inc., Madison Wisconsin 53704-2592 for APME Ad-hoc APFO Toxicology Working Group. Study No. Covance 6329-230, Completion Date December 18, 2001, 159 pp. US EPA AR226-1052a..

Thomford, P.J. 2001b 26-Week capsule toxicity study with ammonium perfluorooctanoate (APFO) in cynomolgus monkeys. Study performed by Covance Laboratories Inc., Madison Wisconsin 53704-2592 for APME Ad-hoc APFO Toxicology Working Group. Study No. Covance 6329-231, Completion Date December 18, 2001, 463 pp. US EPA AR226-1052a..

Trosko, J.E.; Chang, C.C.; Upham, B.L. and Wilson, M. 1998 Epigenetic toxicology as toxicant-induced changes in intracellular signaling leading to altered gap junctional intercellular communication. Toxicol. Lett. 102-103:71-78.

Tugwood, J.D.; Holden, P.R.; James, N.H.; Prince, R.A. and Roberts, R.A. 1998 A PPAR alpha cDNA cloned from guinea pig liver encodes a protein with similar properties to the mouse PPAR alpha: Impricatons for species differences in response to peroxisome proliferators. Arch. Toxicol. 72:169-177.

Ubel, F.A.; Sorenson, S.D. and Roach, D.E. 1980 Health status of plant workers exposed to fluorochemicals--a preliminary report. Am. Ind. Hyg. Assoc. J. 41:584-589.

Upham, B.L.; Deocampo, N.D.; Wurl, B. and Trosko, J.E. 1998 Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Intl. J. Cancer 78:491-495.

U.S. EPA 1991 Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798-63826.

U.S. EPA 1999 Guidelines for carcinogen risk assessment [review draft] MCEA-F-)644. Risk Assessment Forum, Washington, DC. Available from <http://www.epa.gov/ncea/raf/cancer.htm>

U.S. EPA 2002a Memorandum from Dr. Ralph Cooper, NHEERL, to Dr. Jennifer Seed, dated October 2, 2002.

U.S. EPA 2002b Memorandum from Dr. Elizabeth Margosches to Dr. Katherine Anitole, dated October 21, 2002.

U.S. EPA 2003 Letter from Robert A. Bilott to Richard H. Hefter, dated July 3, 2003. U.S. EPA AR226-1372; 8EHQ-0703-00373.

Vanden Heuvel, J.P.; Kuslikis, B.I.. and Peterson, R.E. 1991a Covalent binding of perfluorinated fatty acids to proteins in the plasma, liver and testes of rats. Chem-Biol. Interact. 82:317-328.

Vanden Heuvel, J.P.; Kuslikis, B.I.; Van Rafelghem, M.L. and Peterson, R.E. 1991b Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. J. Biochem. Toxicol. 6(2):83-92.

Vanden Heuvel, J.P.; Davis, J.W.; Sommers, R. and Peterson, R.E. 1992 Renal excretion of perfluorooctanoic acid in male rats: Inhibitory effect of testosterone. J. Biochem. Toxicol. 7(1):31-36.

Walrath J. and Burke, C. 1989 An investigation into the occurrence of leukemia at Washington Works. E. I. Dupont De Nemours and Company, April 1989. US EPA AR226-1308-1.

Welter, A.N. 1979 Technical report summary - final comprehensive report: FC-143. 3M Company. U.S. EPA AR-226 528.

Wolter, J. 1993 Impinger studies of volatility of FC-95 and FC-143. St. Paul, MN. 3M Environmental Laboratory. Lab Request Number L3306. U.S. EPA AR 226-1030a087.

Yager, J.D. Jr. and Yager, R. 1980 Oral contraceptive steorids as promoters of hepatocarcinogenesis in female Sprague-Dawley rats. Cancer Res. 40:3680-3685.

Yang, Q., Xie, Y. and Depierre, W. 2000 Effects of peroxisome proliferators in the thymus and spleen of mice. Clin. Exp. Immunol. 122:219-226.

Yang, Q.; Xie, Y.; Ericksson, A.M.; Nelson, B.D. and DePierre, J.W. 2001 Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. Biochem. Pharmacol. 62:1133-1140.

Yang, Q.; Xie, Y.; Alexson, S.E.H.; Nelson, B.D. and DePierre, J.W. 2002a Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodilation caused by peroxisome proliferators in mice. Biochem. Pharmacol. 63:1893-1900.

Yang, Q.; Abedi-Valugerdi, M.; Xie, Y.; Zhao, X.; Moller, G.; Nelson, B.D. and DePierre, J.W. 2002b Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. Internat. Immunopharmacol. 2:389-397.

Ylinen, M.; Hanhijarvi, H.; Jaakonaho, I. and Peura, P. 1989 Stimulation by estradiol of the urinary excretion of perfluorooctanoic acid in the male rat. Pharmacol. Toxicol. 65:274-277.

Ylinen, M.; Kojo, A.; Hanhijdrvi, H. and Peura, P. 1990 Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. Bull. Environ. Contam. Toxicol. 44:46-53.

York, RG. 200; Oral (gavage) two-generation (one litter per generation) reproduction study of ammonium perfluorooctanoic (APFO) in rats. Argus Research Laboratories, Inc. Protocol Number: 418-020, Sponsor Study Number: T-6889.6, March 26, 2002. U.S. EPA AR226-1092.

Appendix A: Predicting serum concentrations in rats

A-1.0. Introduction

Analysis of pharmacokinetics for PFOA could be done using a number of approaches including physiologically based pharmacokinetic (PBPK) modeling, classical compartmental modeling, and non-parametric analysis. Each of these has strengths and limitation given the available data. Some of the literature and submitted pharmacokinetic studies have been analyzed using non-parameteric analyses. This provides a description of the data that have been collected, but has fairly limited ability to make predictions across species or variations in exposures. Classical compartmental modeling can be used to analyze the existing data on blood concentrations in rats, monkeys, and humans. Much of the published analyses use this approach and the data in humans and perhaps monkeys is only adequate to support this approach. Comparisons of serum protein binding across species indicated a high degree of binding in all species eliminating the apparent need to address this factor in the compartment modeling. PBPK modeling is perhaps the ideal approach for addressing PFOA for purposes of cross-species extrapolation. Extensive pharmacokinetic studies have been undertaken in rodents demonstrating complex phenomenon such as high tissue concentrations in liver, kidney and serum and enterohepatic recirculation of the parent compound. These could be addressed using PBPK modeling for the rodents, but the more limited information in monkeys and humans would either require substantial assumptions or preclude use of this approach. In light of the documented differences in clearance of PFOA across sexes in rats and across species, compartmental modeling of serum concentrations provides a sound approach for estimating internal dosimetry without exceeding the limits of the available data, so this approach was selected for this risk assessment.

The calculations (see Sections 5.4 and 5.5) of margins of exposures (MOEs) using human and rodent plasma concentrations or the Area Under Concentration curve in plasma (AUC) require either direct measurements or prediction of these concentrations and AUCs. AUC is a measure of dose that reflects both concentration and time; i.e., it increases with higher concentrations and longer durations. The AUCs estimated here are the average daily AUC under conditions of repeated dosing. Concentrations in plasma at the time of measurement reflect the time course for circulating chemical including the maximum levels achieved following a bolus dose; with slow clearance and repeated dosing they can reflect steady state conditions. Pharmacokinetic studies in Sprague-Dawley rats (Kemper (2003); Han (2003); see Section 3.2) provided information for parameterizing and calibrating a predictive pharmacokinetic model using standard software (WinNonlin® Version 4.0.1). Measurements of plasma concentrations in several other studies with Sprague-Dawley rat (York, 2002; Mylchreest, 2003; Palazzolo, 1993; Goldenthal, 1978a) provide data for evaluating the model (see Section A-4.0) and in some cases for direct comparison to human measured plasma concentrations in Section 5.5.

Compartmental models were fitted to the data for individual adult animals in Kemper (2003) (see Section A-5.0). WinNonlin® Version 4.0.1 was applied to oral plasma time course data to estimate and compare one-compartment and two-compartment models with both absorption and elimination constants. Similar fitting was done for the intravenous plasma time course data. Akaike Information Criteria and correlations of observed and predicted values at each dose holistically afforded a basis for the comparisons of the fits and the one- and two-compartment models. For either sex, in the range of dosing of most interest, there was little difference among the models, although there is a large difference in elimination rate between adult male and female rats.

A one-compartment model was selected for predicting plasma concentrations and AUCs due to its ease of use and consistency with the data. The plasma concentration in such a model with first-order oral absorption and excretion can be expressed (see O'Flaherty, 1981, equation 3.42) as:

$$C = \frac{k_a D}{V(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}).$$

where C is the plasma concentration at a given time (mg/L), D is the dose (mg), V is the volume of distribution (L), k_a is the absorption rate constant (/hr), k_e is the elimination rate constant (/hr), and t is time (hr). Then AUC = $D/(Vk_e)$; this is expressed in mg-hr/L or equivalently, ug-hr/mL. Sex-specific averages of V, k_e and k_a were calculated across the one-compartment model fits to all the orally-administered doses. Further, AUC can be expressed in terms of the frequently reported body-weight-adjusted values for dose and volume of distribution. D can be expressed as D = body weight x δ, where δ is the administered dose rate in mg/kg per day, and V can be written V = body weight x β, where β is a per-unit-weight value in L/kg. Thus, AUC = $\delta/(\beta k_e)$, also in mg-hr/L or ug-hr/L.

Table A-i Pharmacokinetic Model Values for Adult Rats

	β (L/kg)	k_a (/hr)	k_e (/hr)	$t_{1/2}$ (hr)
Male Rat	0.172	3.87	0.0058	119
Female Rat	0.168	3.73	0.216	3.2

Estimates of AUC were determined for the doses, exposure durations, and life stages relevant to the toxicity studies for which calculations were planned for Section 5. For gavage dose studies, the oral dose received by the animals was known. For dietary studies, the daily dose was estimated based upon food consumption and chemical concentration in the diet. AUCs using the parameter values as described above were predicted for toxicity studies involving adult male and nonpregnant female rats. The two-generation reproductive toxicity study includes males, pregnant females, lactating females, developing fetuses, lactationally exposed offspring, and juvenile offspring, so estimates of AUC are desirable for each of these lifestages. Estimates of AUC for gavage-dosed males, pregnant females, and lactating females used the parameter values as described above. As indicated in Section A-4.0, plasma data from pregnant and lactating females were reasonably predicted by the modeling without adjustments for these lifestages. Owing to the evidence in the Han (2004) study with juvenile animals that the clearance in males drops dramatically with sexual maturation after week 4, these data were used to re-estimate weekly values of k_e for gavage dosed juvenile males and females. No estimates for AUC for lactationally-exposed offspring were developed owing to uncertainties in the dose they received, as discussed in Section 5.

A-2.0. Adult Male and Female Rats

A-2.1. Adult Male Rats

Based on Kemper (2003), adult male rats have relatively slow clearance, with average half life just under a week (about 5 days). It takes 21 days to be within 95% of steady state levels. Consequently, adult male rats in toxicity studies longer than 21 days would be expected to be at steady state, though neither the single dose nor the extended course pharmacokinetic studies would have achieved steady state. Data from the subchronic toxicity study by Palazzolo (1993),

which extended 14 weeks, can be used to evaluate the adequacy of predictions based upon single doses for repeated dosing of male rats to steady state (see Section A-4.0).

At steady state,

$C_{steady\ state}$ or C_{SS} = *(dose rate mg/kg)/((male* k_e */hour)*(male* β *L/kg)*(24hour/day))*
AUC= *24*(conc at steady state) = 24**C_{SS}

Table A-ii.a. Values for Adult Male Rats

administered dose (mg/kg)	1	3	10	30
AUC (μg-hr/mL)	1011	3032	10108	30324
C_{SS} (mg/L)	42	126	421	1263

A-2.2. Adult Female Rats

Females have rapid clearance, with an estimated half-life of 0.134 days or 3.2 hours. The female will clear virtually the entire gavage or dietary dose each day, so plasma concentrations will vary throughout the day, but successive days will appear similar. The process does not reach steady state, but an AUC can be estimated.

Table A-ii.b. Values for Adult Female Rats

administered dose (mg/kg)	1.6	3	10	30
AUC (μg-hr/mL)	44.16	82.80	276.01	828.03

A-3.0. Post-weaning, Juvenile Rats

To estimate AUCs for the gavage dosed juvenile rats in the two-generation toxicity study, the data from Han (2003) were used to estimate k_e in a week- and sex-specific manner. Supposing the adult per-unit-weight volume of distribution β estimated from Kemper (2003) holds for the offspring, the elimination constant can be obtained from the Han (2003) data using

$$C(T)=(D/V)(\exp(-k_eT))=(\delta/\beta)(\exp(-k_eT))$$

where $C(T)$ is the concentration at 24 hours after gavage dosing, δ = 10 mg/kg as used in Han (2003), and β is the adult sex-specific weight-adjusted volume of distribution from the WinNonlin® solutions to Kemper (2003). Absorption is long completed by 24 hours, so an instantaneous absorption assumption is not very influential. That is, the concentration equation above is essentially the same as the one in Section A-1.0, when k_a is large relative to k_e. In Section A-5.0 it will be seen k_a/k_e is about three orders of magnitude for male adult rats and one and a half for female adults. The impact of their relative size has been further verified for the rats by successfully re-estimating the 24-hour plasma concentrations of Han (2003) using the expanded equation, the weekly elimination rates, and the adult absorption rates (results not shown).

Then,

$$k_e /hr = (-\log_e(\beta * C(T))/\delta)/T$$
$$= (-\ln(\beta\ L/kg * (C(24\ hr)\ mg/L)))/\delta\ mg/kg)/(24\ hr)$$

This sex-specific k_e is then used with the equation AUC = $D/(Vk_e) = (\delta/(\beta k_e))$ to obtain the AUCs.

A-3.1 Post-weaning, Juvenile Male Rats

For the males, half lives range from a third of a day to 2.5 days throughout the post-weaning period of four to eight weeks of age in Han (2003). The longer half-life observed from weeks 5 to 8 would require 10 days to be within 95% of steady state. Treating the juvenile males as if they are at steady state is, thus, an approximation till steady state would be achieved around week 6 or 7.

$$AUC = 24\ hr * \delta\ mg/kg/day\ /(\beta\ L/kg * k_e\ /hr * 24\ hr/day)$$

Table A-iii. Post-weaning Male Rats, AUC, using 0.172 = adult male β

dose rate	1	3	10	30
4wk AUC (µg-hr/mL)	67.40	202.19	673.96	2021.87
5wk AUC (µg-hr/mL)	354.25	1062.76	3542.53	10627.59
6wk AUC (µg-hr/mL)	468.13	1404.39	4681.29	14043.85
7wk AUC (µg-hr/mL)	310.66	931.98	3106.62	9319.84
8wk AUC (µg-hr/mL)	339.11	1017.32	3391.07	10173.20
mean wks4-8	307.91	923.73	3079.09	9237.27

A-3.2. Post-weaning, Juvenile Female Rats

For the females, k_e estimated in a week-specific manner from Han (2003) can also be applied. A sex-specific k_e is again used with the equation AUC = $D/(Vk_e) = (\delta/(\beta k_e))$ to obtain the AUCs. Half-lives of the female pups range about 3.5-5.5 hours, so steady state would not be achieved following gavage dosing.

$$AUC = \delta\ mg/kg\ /\ (\beta\ L/kg * k_e\ /hr)$$

Table A-iv. Post-weaning Female Rats, AUC, using 0.168 = adult female β

dose (daily)	1	3	10	30
4wk AUC (µg-hr/mL)	46.11	138.33	461.11	1383.34
5wk AUC (µg-hr/mL)	36.07	108.21	360.69	1082.06
6wk AUC (µg-hr/mL)	36.47	109.40	364.67	1094.01
7wk AUC (µg-hr/mL)	30.76	92.28	307.60	922.81
8wk AUC (µg-hr/mL)	33.28	99.82	332.75	998.25
mean wks4-5	41.09	123.27	410.90	1232.70

A-4.0. Validation of One-compartment Model for Predicting Dose Metrics

The parameters for the one-compartment pharmacokinetic model applied to PFOA were derived from fits using WinNonlin®4.0.1 with single oral dose pharmacokinetic studies in male and female Sprague-Dawley rats (Kemper 2003). These studies measuring plasma time courses used doses of 0.1, 1 (two different studies with different durations), 5, and 25 mg/kg. The average parameter values across these five data sets were used when making predictions. A

single intravenous dose (1 mg/kg) in male and female Sprague-Dawley rats provides independent estimates of the volume of distribution and elimination rate that are reasonably consistent with the average values from the oral studies (Kemper, 2003). Another Kemper (2003) study in male and female Sprague-Dawley rats showed no major changes in the extent of total urinary and fecal elimination for a 1 mg/kg oral dose given following 14 days of dosing at 1 mg/kg/day (i.e., there were no apparent pharmacokinetic changes due to repeated dosing; see Section 3.2.3.5.2.1). Additional urinary and fecal elimination studies following single doses are available, but have not yet been subjected to quantitative analysis using the one-compartment model.

The parameters for males and females can be used to predict blood plasma concentrations that were measured in several of the toxicity studies. Plasma levels following gavage dosing also have been reported for adult female rats in the tissue distribution studies of pregnant rats and lactationally exposed offspring (Mylchreest, 2003). Parental male and female Sprague-Dawley rats in the 2-generation study had blood sampled at the end of mating and lactation, respectively (York, 2002). Two dietary studies exposed rats for 90 days at the end of which blood samples were obtained. One study used male Sprague-Dawley rats (Palazzolo, 1993). The other used male and female Sprague-Dawley rats (Goldenthal, 1978a).

Different analyses are required for predicting the plasma concentrations in these studies using the one-comparment model. Owing to rapid clearance in females, it is important to predict the concentration at the appropriate time following dosing, even when it is measured in a repeated dosing study. Modeling dietary exposure for females is likely to be somewhat problematic using a simple bolus approach. For males, their relatively slow clearance would result in achieving steady state plasma levels following sufficient duration of dosing; it should make little difference whether the exposure was by gavage or diet.

The plasma concentrations in pregnant and lactating rats 2 hrs after dosing are reasonably well predicted using the average values from the oral pharmacokinetic studies in females for the volume of distribution, elimination rate, and oral absorption rate (Mylchreest, 2003). The predicted concentrations at the higher doses exceed the measured levels by less than a factor of two, while the lower dose is well predicted. Small differences in the kinetic parameters would be expected for pregnant animals; pregnancy might lower their predicted plasma concentrations (owing to a larger volume of distribution, reduced protein binding and increased clearance). Lactating females eliminate some fraction of their body burdens in their milk, which may also reduce their plasma concentrations relative to these predictions.

Table A-v. Predicting Mylchreest (2003) 2-hour dam plasma concentrations (gavage)*

Time (hr)	Daily Dose (mg/kg)	predicted C(2hr) (ug/mL)	measured (ug/mL)	measured SD
2	3	12.29	11.19	2.76
	10	40.96	26.84	4.21
	30	122.87	66.64	9.8

**C(2hr)* calculated from

$$C(t) = \frac{k_a D}{V(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}) = \frac{k_a \delta}{\beta(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}).$$

The plasma concentrations 24 hrs following gavage dosing of lactating female rats also are reasonably well predicted at both the 10 and 30 mg/kg/day dose levels (York, 2002).

Table A-vi. Predicting York (2002) 24-hr dam plasma concentrations (gavage)*

Time (hr)	Daily Dose (mg/kg)	predicted C(24 hr) (ug/mL)	measured (ug/mL)	measured SD
24	10	0.35	0.37	0.0805
	30	1.06	1.02	0.425

**C(24hr)* calculated from

$$C(t) = \frac{k_a D}{V(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}) = \frac{k_a \delta}{\beta(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}).$$

Steady state plasma concentrations in male rats exposed via diet for 90 days were predicted within a factor of two at a wide range of doses (Palazzolo, 1993).

Table A-vii. Predicting Palazzolo (1993) male plasma concentrations (diet)*

Time (hr)	Diet (ppm)	Dose Rate (mg/kg/day)	Dose Rate SD (mg/kg/day)	predicted C_{ss} (ug/mL)	measured avg 5, 8, 14 wks (ug/mL)
24	1	0.06	0.013	3	7.0
	10	0.64	0.138	27	47.4
	30	1.94	0.408	82	87.0
	100	6.5	1.214	274	148.7

*calculated as C_{SS} *(mg/L) = (dose rate mg/kg/day)/((β L/kg)*(k_e /hour)*(24hour/day))*

These data and predicted levels provide confidence that the model using parameters independently determined in the pharmacokinetic study is able to predict dose metrics in the toxicity studies.

There are two groups of data that are not successfully predicted, the male plasma concentrations from the York (2002) and Goldenthal (1978a) studies. It is important to note that the measured concentrations in these studies are not consistent with the results of the Palazzolo (1993) study, so it would be impossible for a single set of parameter values to predict all these data sets. The source of this difference in measured results is unclear, particularly for the York (2002) results since the female data from that study appear reasonably predicted. The analysis of plasma from the Goldenthal (1978a) study involved considerably different analytical methods, which may be an important factor.

The York (2002) male data reports plasma concentrations at 10 and 30 mg/kg/day that are similar to those observed at 0.6 and 1.9 mg/kg/day (estimated for dietary exposure) in the Palazzolo (1993) study. Treating the data as concentrations 24 hrs following a single gavage gives a reasonable prediction for the lower dose, but not the higher one. Average concentrations at 24 hr following a single oral dose of 25 mg/kg in Kemper (2003) are 127 ug/mL, clearly inconsistent with the 45 ug/mL plasma level measured following repeated gavage in York (2002) males. The males in York (2002) were being mated, but the potential for that to contribute to changes in pharmacokinetics is unclear.

Table A-viii. Predicting York (2002) 24-hr male plasma concentrations (gavage)*

Time (hr)	Dose Rate (mg/kg/day)	predicted Css (ug/mL)	measured (ug/mL)	measured SD	predicted C(24hr) (single dose) (ug/mL)
24	10	421	51.1	9.3	50.77
	30	1263	45.3	12.6	152.31

*calculated as C_{SS} *(mg/L) = (DR mg/kg/day)/((β L/kg)*(k_e /hour)*(24hour/day))* and at 24 hr from a single dose as

$$C(t) = \frac{k_a D}{V(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}) = \frac{k_a \delta}{\beta(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}).$$

The plasma data in male rats exposed by diet for 90 days should similarly represent pseudo-steady state concentrations (Goldenthal, 1978a). Plasma from these animals was analyzed and reported separately by Belisle (1978). While the plasma levels at the lowest dietary exposure are well predicted, the measured plasma values at higher dietary levels show little increase with dose and are not well predicted.

Table A-ix. Predicting Goldenthal (1978a) male plasma concentrations (diet)*

Time (hr)	Diet (ppm)	Daily Dose (mg/kg)	predicted Css (ug/mL)	measured (ug/mL)
24	10	0.6	25	21
	30	1.7	72	34
	100	5.6	236	36
	300	17.9	754	38
	1000	63.5	2674	49

*calculated as C_{SS} *(mg/L) = (dose rate mg/kg/day)/((β L/kg)*(k_e /hour)*(24hour/day))*

The plasma data from female rats exposed by diet for 90 days are more difficult to predict owing to the rapid clearance and the uncertainty in when the rats ate, so these data have not been considered.

TABLES

A-5.0. Results of the WinNonlin® Analyses

A-5.0. Results of the WinNonlin® Analyses

The WinNonlin® solutions corresponding to V, k_a and k_e are denoted in its output by V_F, K01, and K10, respectively, for the 1-compartment model (additional different coefficients are estimated for a 2-compartment model as shown in tables in A-5.0 for these analyses).

Table A-x. Female Rats, One-Compartment Model

Variable	N	Nmiss	Nobs	Mean	SD	SE	Variance	Min	Median	Max	Range	CV%	Geometric	Harmonic M	Pseudo SD	Mean Log	SD Log	CV% Geom	Skewness	Kurtosis
0.1 mg/kg female																				
V_F	3	0	3	28.31038	0.953683	0.550609	0.909511214	27.26332	28.5385	29.12932	1.865992	3.368669	28.29959	28.28871	0.970518	3.342847	0.033893	3.390233	-0.414295	-1.5
K01	3	0	3	6.522584	1.525262	0.88061	2.326424021	4.821422	6.978249	7.768082	2.94666	23.38432	6.393595	6.257274	1.778985	1.855297	0.250227	25.41958	-0.499847	-1.5
K10	3	0	3	0.190299	0.035546	0.020522	0.001263511	0.151857	0.19706	0.221978	0.070121	18.67902	0.187981	0.18559	0.038933	-1.671412	0.194163	19.60074	-0.336812	-1.5
BW	4			176.4	8.6															
beta (L/kg)				0.16049																
0.1 mg/kg female (extended time course)																				
V_F	4	0	4	26.85968	5.712687	2.856344	32.63479772	21.29838	25.76741	34.60553	13.30716	21.26863	26.42605	26.01866	5.13963	3.27435	0.206269	20.84821	0.567924	-1.079306
K01	4	0	4	1.731362	0.732875	0.366437	0.537105296	1.034098	1.717244	2.456863	1.422765	42.32936	1.61078	1.498008	0.656779	0.476718	0.444473	46.73563	0.017135	-1.93209
K10	4	0	4	0.221401	0.042599	0.021299	0.001814668	0.172791	0.220222	0.272367	0.099576	19.24064	0.218303	0.215218	0.042948	-1.52187	0.194633	19.64913	0.078793	-1.319645
BW	4			201	11.8															
beta (L/kg)				0.13363																
1.0 mg/kg female intravenous																				
V (mL)	4	0	4	44.7764	4.3264	2.1632	18.7175	41.0724	43.8474	50.3385	9.2661	9.6622	44.623	44.4733	4.1263	3.7982	0.0952	9.5431	0.4393	-1.4204
K10 (1/hr)	4	0	4	0.1703	0.029	0.0145	0.0008	0.1391	0.1713	0.1995	0.0603	17.0288	0.1684	0.1665	0.0291	-1.7814	0.1726	17.3878	-0.0523	-1.7964
BW	4			200.6	2.7															
beta (L/kg)				0.223212																
1.0 mg/kg female oral																				
V_F	4	0	4	35.77252	7.683779	3.841889	59.04045812	24.93616	37.81364	42.52666	17.59049	21.47955	35.07346	34.30106	9.588217	3.557445	0.237019	24.03874	-0.77394	-0.951693
K01	4	0	4	4.989439	2.068961	1.034481	4.280600063	2.599878	5.147627	7.062624	4.462746	41.46681	4.633671	4.272174	2.298085	1.533349	0.45924	48.45517	-0.147969	-1.656947
K10	4	0	4	0.161888	0.0214	0.0107	0.000457953	0.148536	0.152607	0.193803	0.045267	13.21891	0.160907	0.160005	0.018132	-1.826932	0.124934	12.54231	1.115285	-0.697342
BW	4			197	9.7															
beta (L/kg)				0.181586																
5 mg/kg female oral																				
V_F	4	0	4	31.74406	13.54995	6.774977	183.6012501	18.41039	30.6327	47.30047	28.89008	42.685	29.53732	27.48214	12.25893	3.385655	0.443016	46.56689	0.14896	-1.689577
K01	4	0	4	2.152185	1.982879	0.99144	3.931809422	0.412166	2.072876	4.050822	3.638655	92.13331	1.302084	0.787231	0.794923	0.263966	1.256424	196.1674	0.017262	-1.975965
K10	4	0	4	0.334105	0.121041	0.06052	0.014650842	0.230922	0.31729	0.470918	0.239996	36.22836	0.317682	0.302361	0.107012	-1.146704	0.367695	38.04798	0.16179	-1.786693
BW	4			187.3	3.6															
beta (L/kg)				0.169482																
25 mg/kg female oral																				
V_F	4	0	4	37.75743	9.235502	4.617751	85.29449457	24.25588	41.51663	43.74058	19.48471	24.46009	36.74818	35.58839	12.54934	3.604089	0.281402	28.7066	-0.993507	-0.815105
K01	4	0	4	3.941804	1.961216	0.980608	3.846368692	2.886762	2.99903	6.882396	3.995634	49.75427	3.65612	3.452334	1.045302	1.296402	0.422148	44.16727	1.15179	-0.668879
K10	4	0	4	0.171864	0.009952	0.004976	9.90461E-05	0.159052	0.174024	0.180355	0.021303	5.79074	0.171644	0.171421	0.010261	-1.762331	0.058639	5.868926	-0.443669	-1.416326
BW				194.8	14.4															
beta (L/kg)				0.193827																

Table A-xi. Female Rats, Two-Compartment Model

Variable	N	Nmiss	Nobs	Mean	SD	SE	Variance	Min	Median	Max	Range	CV%	Geometric Mean	Harmonic Mean	Pseudo SD	Mean Log	SD Log	CV% Geometric Mean	Skewness	Kurtosis
1 mg/kg oral																				
V1_F	4	0	4	18.6234	21.3178	10.6589	454.4486	0.1737	16.9581	40.4038	40.2301	114.4676	3.2585	0.5067	1.4802	1.1813	2.8296	5476.9987	0.0528	-1.9296
V2_F	4	0	4	9.0363	6.1941	3.0970	38.3667	2.0499	9.2846	15.5262	13.4762	68.5465	6.9706	4.9841	7.8707	1.9417	0.9196	115.2987	-0.0805	-1.6551
K01	4	0	4	2.7225	2.7532	1.3766	7.5800	0.3420	2.2161	6.1158	5.7739	101.1273	1.4950	0.8091	1.2047	0.4021	1.3888	242.4962	0.3257	-1.5664
CL_F	4	0	4	6.0166	1.7649	0.8825	3.1150	3.9358	5.9444	8.2420	4.3062	29.3344	5.8175	5.6171	1.8777	1.7609	0.3036	31.0689	0.1398	-1.0179
CLD2_F	4	0	4	22.5062	15.8217	7.9109	250.3273	9.7486	17.3694	45.5376	35.7890	70.2993	19.0904	16.6218	10.1250	2.9492	0.6438	71.6701	0.9516	-0.8009
5 mg/kg oral																				
V1_F	3	0	3	29.4424	15.5140	8.9570	240.6835	18.7050	22.3928	47.2294	28.5244	52.6926	27.0454	25.1483	10.1302	3.2975	0.4911	52.2281	0.6624	-1.5000
V2_F	3	0	3	15.4949	20.3798	11.7663	415.3361	3.0245	4.4472	39.0132	35.9887	131.5255	8.0658	5.1624	4.3887	2.0876	1.3786	238.5370	0.7032	-1.5000
K01	3	0	3	1.6408	2.0681	1.1940	4.2771	0.4126	0.4814	4.0285	3.6160	126.0400	0.9283	0.6317	0.4303	-0.0743	1.2735	201.5338	0.7062	-1.5000
CL_F	3	0	3	9.3461	1.1661	0.6733	1.3598	8.4231	8.9586	10.6567	2.2336	12.4771	9.2992	9.2540	1.0768	2.2299	0.1220	12.2418	0.5431	-1.5000
CLD2_F	3	0	3	0.2948	0.0961	0.0555	0.0092	0.1958	0.3009	0.3877	0.1919	32.5940	0.2837	0.2725	0.1066	-1.2598	0.3453	35.5869	-0.1164	-1.5000
25 mg/kg oral																				
V1_F	4	0	4	37.9011	8.5209	4.2605	72.6061	25.4681	41.1476	43.8412	18.3732	22.4820	37.0614	36.1080	11.2420	3.6126	0.2546	25.8795	-0.9811	-0.8231
V2_F	4	0	4	81.1708	126.4525	63.2263	15990.2410	3.0616	26.0788	269.4641	266.4025	155.7857	25.4384	9.2358	30.9321	3.2363	1.8883	586.2240	1.1056	-0.7073
K01	4	0	4	13.3420	18.3460	9.1730	336.5755	2.7921	4.9360	40.7041	37.9120	137.5052	6.9181	4.5843	3.4425	1.9341	1.2556	195.9054	1.1165	-0.6990
CL_F	4	0	4	6.3948	1.4077	0.7039	1.9816	4.3052	6.9487	7.3764	3.0712	22.0134	6.2576	6.1006	1.8870	1.8338	0.2509	25.4927	-1.0813	-0.7155
CLD2_F	4	0	4	0.0520	0.0517	0.0259	0.0027	0.0022	0.0421	0.1218	0.1196	99.3637	0.0254	0.0078	0.0620	-3.6724	1.7350	439.1947	0.5552	-1.1206
LT	4	0	4	0.0599	0.1197	0.0598	0.0143	0.0000	0.0001	0.2395	0.2395	199.6773	0.0004	0.0000	0.0002	-7.7893	4.4339	1857561.7895	1.1547	-0.6667

Table A-xii. Female Rats, Comparisons of One- and Two-Compartment Models
Individual Animal Results within Dose Groups

	one compartment		two compartment	
0.1 mg/kg female	Correlation (obs *vs* pred)	AIC	Correlation (obs *vs* pred)	AIC
	0.94	-15.90436		
	0.955	-18.71725		
	0.9463	-13.88521		
0.1 mg/kg female (extended time course)				
	0.9748	-22.51555		
	0.9946	-32.64225		
	0.9965	-30.0737		
	0.9914	-27.13124		
1.0 mg/kg female intravenous				
	0.9896	-2.59243		
	0.9847	6.47658		
	0.9748	5.75399		
	0.9538	14.09414		
1.0 mg/kg female oral				
	0.9654	13.33264	0.9952	-1.90618
	0.9826	4.95944	0.9828	8.82158
	0.9952	3.2396	0.9981	-3.38119
	0.9949	-7.62189	0.9949	-3.64701
5 mg/kg female oral				
	0.9868	40.17303	0.9846	41.67803
	0.9846	37.67916	0.9815	40.29375
	0.9816	36.31119	0.9947	30.01372
	0.9946	26.33912		
25 mg/kg female oral				
	0.9348	123.12264	0.9639	121.62356
	0.9641	93.547	0.9641	99.5497
	0.9468	99.72258	0.9467	105.71465
	0.9873	76.97422	0.9873	82.97019

Table A-xiii. Male Rats, One-Compartment Model

Variable	N	Nmiss	Nobs	Mean	SD	SE	Variance	Min	Median	Max	Range	CV%	Geometric Mean	Harmonic Mean	Pseudo SD	Mean Log	SD Log	CV% Geometric Mean	Skewness	Kurtosis
0.1 mg/kg male																				
V_F	4	0	4	52.9274	10.5147	5.2573	110.5579	44.1028	50.6338	66.3393	22.2364	19.8662	52.1710	51.4522	9.5789	3.9545	0.1945	19.6356	0.4188	-1.4551
K01	4	0	4	5.5522	6.6896	3.3448	44.7506	0.4407	3.5082	14.7519	14.3112	120.4849	2.3561	1.0479	1.5954	0.8570	1.6751	394.2644	0.6881	-1.1415
K10	4	0	4	0.0041	0.0008	0.0004	0.0000	0.0032	0.0041	0.0050	0.0019	18.7383	0.0040	0.0040	0.0008	-5.5153	0.1911	19.2810	0.0136	-1.0632
BW	4			216.3	11.2															
beta (L/kg)				0.245																
0.1 mg/kg male (extended time course)																				
V_F	4	0	4	37.4251	4.3777	2.1888	19.1639	34.0091	36.1936	43.3040	9.2948	11.6971	37.2396	37.0614	4.0574	3.6174	0.1142	11.4535	0.5927	-1.2525
K01	4	0	4	9.1214	4.4205	2.2103	19.5409	3.0705	10.2760	12.8631	9.7927	48.4629	7.9849	6.6324	7.4220	2.0776	0.6600	73.8877	-0.6576	-1.1410
K10	4	0	4	0.0042	0.0009	0.0004	0.0000	0.0035	0.0040	0.0053	0.0018	20.7981	0.0041	0.0041	0.0008	-5.4902	0.2032	20.5287	0.4414	-1.4335
BW	4			272.5	5.8															
beta (L/kg)				0.137																
1.0 mg/kg male intravenous																				
V	4	0	4	32.5522	1.9014	0.9507	3.6153	29.9308	32.8975	34.4829	4.5521	5.8411	32.5096	32.4661	1.9867	3.4815	0.0594	5.9472	-0.6005	-0.9207
K10	4	0	4	0.0106	0.0015	0.0008	0.0000	0.0085	0.0109	0.0121	0.0036	14.2809	0.0105	0.0104	0.0017	-4.5533	0.1502	15.1057	-0.6236	-0.9151
BW	4			248.3	9.3															
beta (L/kg)				0.131																
1.0 mg/kg male oral																				
V_F	4	0	4	36.9875	5.0695	2.5348	25.6999	33.5521	34.9621	44.4738	10.9217	13.7060	36.7458	36.5234	4.3194	3.6040	0.1298	13.0362	1.0518	-0.7523
K01	4	0	4	1.7823	1.7370	0.8685	3.0173	0.6855	1.0358	4.3723	3.6868	97.4575	1.3350	1.0983	0.6166	0.2890	0.8188	97.7228	1.1136	-0.6980
K10	4	0	4	0.0066	0.0025	0.0013	0.0000	0.0050	0.0055	0.0103	0.0054	38.1633	0.0063	0.0061	0.0016	-5.0667	0.3365	34.6264	1.0913	-0.7174
BW	4			248.7	1.6															
beta (L/kg)				0.149																
5 mg/kg male oral																				
V_F	4	0	4	32.3112	4.8117	2.4058	23.1522	26.2113	32.5260	37.9817	11.7704	14.8916	32.0354	31.7538	5.0832	3.4668	0.1524	15.3329	-0.1542	-0.9947
K01	4	0	4	0.6954	0.1078	0.0539	0.0116	0.5956	0.6730	0.8399	0.2444	15.5025	0.6893	0.6836	0.0988	-0.3720	0.1510	15.1834	0.5545	-1.1896
K10	4	0	4	0.0068	0.0017	0.0008	0.0000	0.0049	0.0067	0.0090	0.0040	24.1979	0.0067	0.0065	0.0017	-5.0071	0.2441	24.7813	0.2402	-1.0168
BW	4			218.0	3.4															
beta (L/kg)				0.1482																
25 mg/kg male oral																				
V_F	4	0	4	40.5204	2.9308	1.4654	8.5897	36.8396	40.8789	43.4842	6.6447	7.2329	40.4397	40.3579	3.0151	3.6998	0.0732	7.3309	-0.3107	-1.3840
K01	4	0	4	1.5115	0.6513	0.3256	0.4242	0.6062	1.6718	2.0962	1.4900	43.0894	1.3691	1.1990	1.0492	0.3141	0.5605	60.7491	-0.7038	-1.0216
K10	4	0	4	0.0071	0.0020	0.0010	0.0000	0.0043	0.0075	0.0090	0.0047	27.9451	0.0068	0.0066	0.0026	-4.9859	0.3187	32.6975	-0.6739	-0.8978
BW				225.0	3.7															
beta (L/kg)				0.1801																

Table A-xiv. Male Rats, Two-Compartment Model

Variable	N	Nmiss	Nobs	Mean	SD	SE	Variance	Min	Median	Max	Range	CV%	Geometric Mean	Harmonic Mean	Pseudo SD	Mean Log	SD Log	CV% Geometric Mean	Skewness	Kurtosis
0.1 mg/kg oral																				
V1_F	4	0	4	48.4637	12.2283	6.1141	149.5305	39.3870	44.5696	65.3285	25.9416	25.2318	47.3934	46.4259	10.1673	3.8585	0.2399	24.3361	0.7009	-1.1286
V2_F	4	0	4	11.1415	7.6879	3.8439	59.1033	2.7865	10.1815	21.4164	18.6299	69.0023	8.8677	6.6436	9.8784	2.1824	0.8478	102.5682	0.4231	-0.9616
K01	4	0	4	2.2464	2.0255	1.0127	4.1025	0.4244	2.2807	3.9999	3.5755	90.1638	1.3974	0.8626	0.9233	0.3346	1.2197	185.1179	-0.0023	-1.9969
CL_F	4	0	4	0.2009	0.0606	0.0303	0.0037	0.1265	0.2096	0.2581	0.1317	30.1333	0.1935	0.1856	0.0692	-1.6427	0.3267	33.5623	-0.2982	-1.5302
CLD2_F	4	0	4	0.3049	0.1821	0.0911	0.0332	0.0413	0.3730	0.4324	0.3910	59.7312	0.2224	0.1252	0.4264	-1.5032	1.1290	160.5430	-0.9355	-0.8716
0.1 mg/kg oral extended																				
V1_F	4	0	4	30.3408	9.0159	4.5080	81.2872	19.2578	30.3915	41.3225	22.0646	29.7156	29.2785	28.1789	10.1562	3.3769	0.3149	32.2851	-0.0194	-1.0069
V2_F	4	0	4	19.1722	14.7579	7.3790	217.7959	10.0028	12.7323	41.2217	31.2189	76.9754	16.0769	14.2145	6.0913	2.7774	0.6382	70.9071	1.1270	-0.6864
K01	4	0	4	2.8750	1.1408	0.5704	1.3015	1.2007	3.2665	3.7664	2.5657	39.6809	2.6356	2.3383	1.9543	0.9691	0.5284	56.7537	-1.0019	-0.7601
CL_F	4	0	4	0.1397	0.0497	0.0249	0.0025	0.1064	0.1202	0.2120	0.1056	35.5854	0.1340	0.1293	0.0349	-2.0099	0.3224	33.0970	0.9639	-0.8445
CLD2_F	4	0	4	0.6509	0.9006	0.4503	0.8111	0.1371	0.2337	1.9993	1.8622	138.3527	0.3474	0.2411	0.1712	-1.0573	1.1997	179.3915	1.1417	-0.6768
1 mg/kg intravenous																				
V1	4	0	4	32.7068	2.4987	1.2493	6.2433	30.3884	32.3988	35.6412	5.2527	7.6396	32.6357	32.5654	2.4510	3.4854	0.0760	7.6129	0.2107	-1.6772
V2	4	0	4	64.5419	53.4475	26.7238	2856.6356	34.5031	39.5514	144.5620	110.0588	82.8105	52.7869	46.1275	19.3541	3.9663	0.6770	76.2544	1.1418	-0.6770
CL	4	0	4	0.1751	0.0417	0.0208	0.0017	0.1170	0.1854	0.2128	0.0959	23.7936	0.1709	0.1662	0.0527	-1.7667	0.2648	26.9549	-0.7195	-0.9901
CLD2	4	0	4	0.3502	0.1161	0.0581	0.0135	0.2216	0.3454	0.4883	0.2668	33.1568	0.3354	0.3208	0.1180	-1.0925	0.3436	35.4041	0.1087	-1.4102
1 mg/kg oral																				
V1_F	4	0	4	25.5409	9.9245	4.9622	98.4956	13.2942	26.9683	34.9329	21.6387	38.8573	23.8838	22.1208	12.0959	3.1732	0.4412	46.3571	-0.3050	-1.5179
V2_F	4	0	4	17.3278	10.0791	5.0395	101.5874	4.4310	19.0114	26.8573	22.4263	58.1671	14.1875	10.6781	16.3905	2.6524	0.8207	98.0334	-0.3978	-1.3841
K01	4	0	4	0.9458	1.1459	0.5729	1.3130	0.3389	0.3901	2.6642	2.3253	121.1510	0.6088	0.4732	0.2306	-0.4963	0.9864	128.2850	1.1531	-0.6678
CL_F	4	0	4	0.2136	0.0561	0.0280	0.0031	0.1612	0.2004	0.2922	0.1310	26.2483	0.2085	0.2038	0.0476	-1.5680	0.2499	25.3828	0.7246	-0.9303
CLD2_F	4	0	4	1.1540	1.2031	0.6016	1.4475	0.0953	0.8290	2.8628	2.7675	104.2553	0.6469	0.2979	1.1733	-0.4355	1.4271	258.1437	0.8059	-0.9071
5 mg/kg oral																				
V1_F	4	0	4	25.8013	6.9297	3.4649	48.0208	16.2165	27.7764	31.4357	15.2192	26.8580	24.9914	24.0855	8.9814	3.2185	0.3037	31.0890	-0.7071	-1.1008
V2_F	4	0	4	38.1791	14.8007	7.4004	219.0613	27.9870	32.6645	59.4005	31.4135	38.7665	36.3128	34.7743	10.4622	3.5922	0.3544	36.5867	0.8863	-0.9251
K01	4	0	4	0.5151	0.1483	0.0742	0.0220	0.3092	0.5526	0.6461	0.3369	28.7941	0.4962	0.4749	0.1976	-0.7007	0.3303	33.9511	-0.7046	-1.0405
CL_F	4	0	4	0.1594	0.0324	0.0162	0.0010	0.1184	0.1622	0.1946	0.0761	20.3126	0.1568	0.1541	0.0356	-1.8531	0.2125	21.4907	-0.2609	-1.2287
CLD2_F	4	0	4	0.8826	1.5119	0.7560	2.2860	0.0996	0.1402	3.1503	3.0507	171.3095	0.2802	0.1625	0.1015	-1.2721	1.6211	358.3916	1.1541	-0.6671
25 mg/kg oral																				
V1_F	4	0	4	37.8559	1.6269	0.8135	2.6468	35.5592	38.3425	39.1797	3.6204	4.2976	37.8292	37.8019	1.6942	3.6331	0.0437	4.3679	-0.7976	-0.9871
V2_F	4	0	4	33.0604	23.1410	11.5705	535.5047	11.4257	29.3070	62.2020	50.7763	69.9961	26.8232	21.7307	18.0979	3.2893	0.7691	89.8223	0.3596	-1.4551
K01	4	0	4	1.3515	0.5876	0.2938	0.3452	0.4909	1.5868	1.7415	1.2505	43.4743	1.2093	1.0310	1.0644	0.1901	0.6063	66.6481	-1.0041	-0.8037
CL_F	4	0	4	0.2287	0.0616	0.0308	0.0038	0.1615	0.2362	0.2811	0.1197	26.9140	0.2223	0.2158	0.0626	-1.5038	0.2794	28.4933	-0.1164	-1.8463
CLD2_F	4	0	4	0.2269	0.1410	0.0705	0.0199	0.0985	0.1933	0.4225	0.3240	62.1400	0.1968	0.1721	0.1117	-1.6256	0.6157	67.8954	0.6903	-1.0213

Table A-xv. Male Rats, Comparisons of One- and Two-Compartment Models
Individual Animal Results within Dose Groups

	one compartment			two compartment	
0.1 mg/kg male	Correlation (obs *vs* pred)	AIC		Correlation (obs *vs* pred)	AIC
	0.9157	-64.4		0.9471	-58.6
	0.9605	-83.4		0.9605	-79.4
	0.9272	-53.6		0.9356	-51.2
	0.9497	-64.2		0.9595	-65.4
0.1 mg/kg male (extended time course)					
	0.8177	17.3		0.905	-8.1
	0.9829	-95.7		0.9903	-125.6
	0.9804	-96.8		0.9824	-97.9
	0.9757	-77.8		0.985	-91.55
1.0 mg/kg male intravenous					
	0.79	126.7		0.9367	77.9
	0.956	81.6		0.9696	72.4
	0.9114	91.1		0.914	94
	0.9338	89.7		0.9528	82.7
1.0 mg/kg male oral					
	0.9267	47.7		0.9711	36.2
	0.9266	74.5		0.9649	61.5
	0.9162	67.2		0.9252	69
	0.9413	76.5		0.9366	82.4
5 mg/kg male oral					
	0.9576	136		0.9587	138.2
	0.9399	128.4		0.9659	120
	0.8966	156.6		0.9043	158.6
	0.9668	139.4		0.9708	139.7
25 mg/kg male oral					
	0.973	186		0.9822	180
	0.9815	187.6		0.9857	183
	0.9876	176		0.9877	178.8
	0.9732	184.2		0.9822	178.2